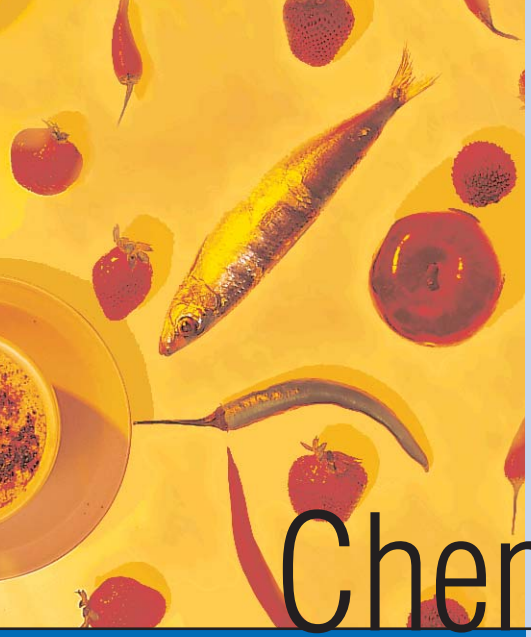
Vol. 6 No.3 June 2004



ChemoSense

EDITORIAL

The How and What of Odorant Delivery

By Graham Bell

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Odorants can reach the olfactory epithelium, where perception begins, by two routes: the normal "forward" path, in through the nose, or the "backward" way, upward through the nasopharynx. Is one's perception equivalent for either route of delivery? Bruce Halpern's review reveals some interesting answers.

The question "what is delivered to the sensors?" has led to a new generation of commercial electronic noses (arrays of chemical sensors). The devices are becoming smaller and are tailored for specific uses. We can foresee thousands of tiny disposable wireless e-noses sewn across wide areas. Linked by satellite communications to superior computing systems, such "arrays of arrays" will provide vital chemical surveillance or monitor polluting chemicals drifting across town or country. Wide Area Chemical Sensing (WACS) is coming.

How much you appreciate a glass of wine, and will pay for it, will depend on the capacity of the wine to deliver its delicious volatiles to your nose. Leigh Francis reports on research into a currently hot topic in the wine industry: part of ongoing competition to maximise the winemaker's contribution to the consumer's pleasure ■

Retronasal and Orthonasal Smelling

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INTRODUCTION

Ortho- is defined as "Correct" (The American Heritage Electronic Dictionary of the English Language, 1992; Flexner, 1987), so orthonasal olfaction is presumably correct olfaction, or at least the process of smelling using the 'correct' nasal approach. What approach is that? Orthonasal stimulus delivery occurs when odorants travel inward from the nostrils (anterior nares) towards the olfactory mucosa during an inhalation or sniff (Figure 1) (DeWeese and Saunders, 1968; Davis, 1980; Pierce and Halpern, 1996; Roberts and Acree, 1995; Voirol and Daget, 1986). Of course, the odorants flow across trigeminally-innervated nasal mucosa along the way (e.g., Bryant and Silver, 2000; Doty and Commetto-Muñiz, 2003; Finger et al., 2003; Silver and Finger, 1991; Shusterman, 2002), so orthonasal smelling can involve both the olfactory system and the trigeminal system. That sounds like a logical, and complete, description of the manner in which smelling begins: odorants dissolved or suspended in air move from the external environment through the anterior nares during inhalations or sniffs, and flow across sensory surfaces.

What purpose or purposes are served by orthonasal smelling? Spors and Grinvald (2002) provide a summary, albeit for olfaction per se: "With every breath, the olfactory receptor neurons in the mammalian nose monitor our chemical environment. Reliable determinations of odor identify and accurate tracking of changes in odorant concentration are important for food localization, social interaction, and prey-predator recognition." The orthonasal-relevant odorous environment envisioned is the

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ISSN 1442-9098

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ambient surround, with perhaps some input from more distant loci.

What is left for retronasal olfaction, and retronasal smelling? The word 'Retro' has its roots in the concept of 'driving or going back' or 'backward' (The American Heritage Electronic Dictionary of the English Language, 1992; Flexner, 1987). Balancing the inward air movement of inhalations or sniffs, there must be exhalations of comparable volume, with the expired, retronasal flow moving air back out of the body. This expiratory flow, which originates in the lungs, may acquire odorants from surfaces and structures that are contacted or intersected as the air moves towards the oral and nasal cavities. Each expiration necessarily passes into the nasopharynx, with subsequent possible routes through the nasal or oral cavities.

If the mouth is closed during an expiration, air flow will be through the nasal cavity, exiting via the anterior nares as a retronasal

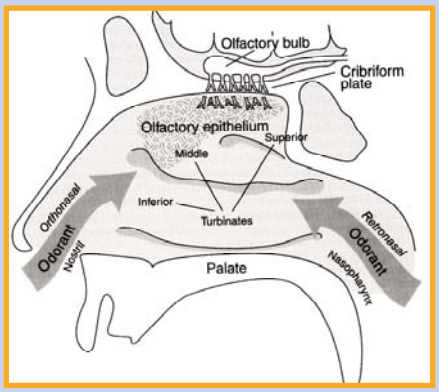

Figure 1. Schematic diagram showing human nostrils, nasal cavity, palate (hard palate to the left; soft, to the right), and nasopharynx. The nasal valve (not shown) is a region of deformable, elastic cartilage not very far (1-2 cm) from the nostril. Much of the nasal cavity is innervated by branches of the trigeminal nerve. Within the nasal cavity, olfactory epithelium is depicted on the upper part of the middle turbinate and on the superior turbinate. The area labeled "Olfactory Epithelium" also represents the narrow cavity called the olfactory cleft. The orthonasal smelling pathway, with odorant entering through the nostril (anterior naris), is shown to the left of the diagram. On the right, the retronasal smelling pathway, with odorant entering from the nasopharynx (i.e., the epipharynx), is depicted. When the velum (far right portion of the soft palate) is down, as shown, a connection between the oral and nasal cavities is available. From Rawson, 2000. This material is used by permission of Wiley-Liss, Inc., a subsidiary of John Wiley and Sons, Inc.

event (Figure 1). Because the oral cavity would be a cul de sac under these circumstances, one might imagine that air-phase (vapor phase) odorants in the oral cavity would neither enter the expiratory stream nor become potential retronasal odorants unless oral cavity-initiated events such as swallowing occurred. Furthermore, because the oral cavity is normally separated from the respiratory passages during part of a swallowing cycle (see Halpern, 2003), one might suspect that the opportunities for oral cavity odorants to become retronasal odorants are very limited.

Explicit proposals for only brief and occasional inputs from the oral cavity into retronasal flow have been made, have been buttressed with laboratory data (Buettner and Schieberle, 2000), and have been incorporated into formal models (Normand et al., 2004). However, substantial data from other sources support an opposite conclusion. They demonstrate that normal expirations without swallowing effectively sample the air-phase component of the oral cavity, that the oral cavity is not separated from the respiratory passages at the start, or termination, of swallowing-related apnea, and that swallowing can diminish the amount of retronasal odorants of oral-origin released during chewing (Halpern, 2003) (Figure 2).

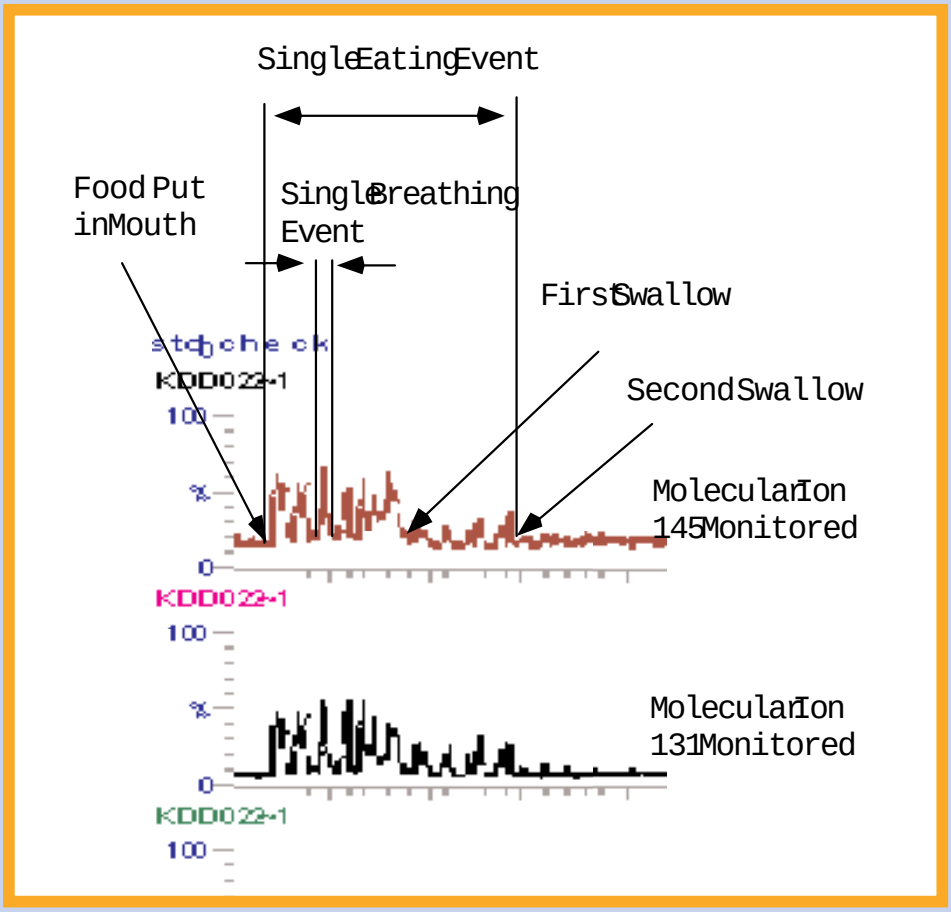

Figure 2. MS-Nose monitoring of ions in the breath of a human while eating an imitation cheese. Molecular ion 145 was ethyl hexanoate; 131 was isoamyl acetate ; and 107, benzaldehyde. All had been incorporated into the imitation cheese. From Deibler, 2001, with permission.

ORTHONASAL AND RETRONASAL ODORANTS

Orthonasal Odorants. Potential odorants for orthonasal smelling comprise the full suite of environmental substances that can dissolve in or be suspended in air, can reach the olfactory epithelium or the nasal or nasopharyngeal mucosa, and can then alter the action potential traffic of the olfactory or trigeminal nerves. Among the sources of

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these orthonasal odorants would be secretions released by intact or damaged animals or plants, including odorants associated with conspecific and sympatric organisms, by food objects, and by predators and prey. Also included would be substances emitted by geophysical events, for example sulfur-containing releases of geothermal processes.

In addition, for humans, substances employed for various purposes, such as insecticides, cleaning preparations, etc. may happen to have smells that serve as markers of the possibly hazardous presence of these substances. Furthermore, odorants arbitrarily coupled for information or warning can be intended to be orthonasal odorants. One example would be providing a smell to a non-odorous but potentially dangerous product, such as methyl or ethyl mercaptan added to methane (What You Need to Know About Natural Gas Detectors, 2004); a second example, a person who intentionally scents themselves to have the olfactory aspects of, perhaps, a flower or tree, after first striving to make themselves non-odorous by bathing.

Often, several or even many members of this large orthonasal odorant set will be present simultaneously, with amount and availability varying over time. The concentrations will frequently be low, and it may be necessary to make relatively rapid decisions based upon the smelled orthonasal array.

Retronasal Odorants. Only a limited subset of the possible orthonasal odorants are likely to be retronasal odorants. Under natural circumstances, the odorant sources must be in the oral cavity. This restricts potential retronasal natural odorant sources to substances that have been taken into the mouth with the intention to ingest or at least to masticate (e.g., chewing gum, beetle leaves), plus substances brought into the oral cavity by defensive or offensive biting, by sexual activity, and by intraoral non-ingestive processing, such as the preparation of leather by chewing (Glory and Honor, 2004). In all cases, and certainly for foods, lingual manipulation and interaction with saliva can result in substantial and sequential differences from the substances that were initially taken into the oral cavity (Roberts and Acree, 1995, 1996).

SENSORY MECHANISMS FOR RETRONASAL AND ORTHONASAL SMELLING

Both orthonasal and retronasal smelling must employ the receptor structures of the olfactory and trigeminal mucosa (Figure 1). Because the same sensory epithelia are used, it might be argued that from a receptor tissue point-of-view retronasal and orthonasal smelling are nothing more than two different routes to odorant stimulation, with at most some difference in ease of access (Pierce and Halpern, 1996) or flow rate (Voirol and Daget, 1986). However, Mozell and his colleagues have pointed out that opposite flow paths of odorants across an sorptive surface necessarily produce reversed spatial-temporal sorption patterns, and that if a homogeneous sensory epithelium were involved, reversed spatial-temporal neural responses might result (Hornung, et al., 1980; Hornung and Mozell, 1985; Mozell, 1970, 1971; Mozell & Jagodowicz, 1973). Differential effects of odorant flow direction on olfactory responses have been referred to as the gas chromatographic model of olfaction (Mozell, 1970; Engen, 1982). Furthermore, instead of being homogeneous, mammalian olfactory epithelia consist of a sizeable number of different receptor types, organized into zones in some species (Dalton, 2002; Korsching, 2002; Ma & Shepherd, 2000; Mori et al., 1999). This selectively sorptive, heterogeneous and perhaps spatially organized olfactory epithelium would necessarily result in different neural response patterns for retronasal versus orthonasal olfaction (Mozell et al., 1987).

Similar effects may occur for trigeminal epithelium. Consequently, the physicochemical and neural aspects of not only the olfactory component of retronasal smelling but also the trigeminal component may differ from those of orthonasal smelling. One necessary qualification is that few of these data are from humans or other primates. It is generally assumed that somewhat comparable structural and functional organizations exists in humans (e.g., Laska, 2004).

ORAL SMELLING

Under natural circumstances, oral odorants are air phase components derived from solids or liquids held and manipulated in the mouth (Figure 2). Characterization of the likely odorants can be obtained from a "Retronasal Aroma Simulator" (RAS) which is designed to approximate the thermal and mechanical events over time inside a typical human mouth (Roberts and Acree, 1995, 1996). There can be a close correspondence between the odorants derived from the RAS and those measured in air exhaled through the nose during chewing (Figure 2). Of course, under *in vivo* conditions, in addition to odorants, the liquid or solid oral stimuli may provide gustatory, thermal, and mechanical stimulation inside the mouth.

Human psychophysical responses to oral stimuli have been explored in numerous investigations, with the majority being intended to examine the role of oral sensory systems such as taste or oral chemesthesis (for reviews see Bartoshuk, 1988; Breslin, 2000; Bryant & Silver, 2000; Halpern, 2002; Lawless, 2000; McBurney, 1978; Pfaffmann et al., 1971). In some instances, nose clips (e.g., Hettinger, et al.,1996; Lawless et al., 2004) or other stimulus restriction methods (e.g., Kelling and Halpern, 1988) were employed to minimize or preclude nasal smelling.

A limited number of studies (e.g., Aubry, et al., 1999; Burdach & Doty, 1987; Burdach, et al., 1984; Cerf-Ducastel & Murphy, 2001, 2003, 2004; Duffy, et al., 1999, 2003; Heilmann, et al,., 2002; Kuo, et al., 1993; Rozin, 1982; Stevens & Cain, 1986) utilized liquids or solids in direct contact with the tongue and other oral tissues, often compared or combined with orthonasal odorant presentations, in order to explore oral smelling. The results of these investigations demonstrated a range of human judgments of these oral odorant and tastants combinations, and interactions with orthonasal odorants. This approach may approximate the natural circumstance that accompanies the drinking of liquids or the mastication of foods in that gustatory, olfactory, somatosensory, and trigeminal stimulation can occur (Gibson, 1966; Roberts and Acree, 1995, 1996). Non-psychophysical human measures such as ERP have confirmed the complex nature of interactions between oral and orthonasal stimulation, and suggest that oral stimulation can act to prime orthonasal responses (Welge-Lüssen, 2004).

RETRONASAL SMELLING

Examination of human retronasal smelling per se, that is, responses to air phase components moving during exhalation from the oral cavity (or nasopharynx) to the nasal cavity and out through the anterior nares (Figure 1), but not accompanied by oral gustatory or thermal stimulation and with minimal oral mechanical stimulation, requires procedures that differ from those that are utilized for studies of "oral smelling", as described in the previous section. Retronasal smelling lacks the richness, ecological validity, and naturalness of oral smelling. However, retronasal smelling is homologous to orthonasal smelling in that only air phase stimuli are involved, flowing across the olfactory and trigeminal mucosa. This permits direct comparisons of judgments of the same odorants delivered by orthonasal or retronasal routes, and allows investigation of the effects of paired retronasal and orthonasal odorants

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Retronasal and Orthonasal Smelling continued

Table 1. Retronasal smelling, oral smelling, and orthonasal smelling thresholds (forced choice), in parts per million (p.p.m.), and suprathreshold intensity judgments for citral and vanillia[1].

ODORANT and MEASURE	Retronasal Smelling[2]	Oral Smelling	Orthonasal Smelling[3]
Citral			
Threshold	0.12	0.005	0.04
Intensity intersect and slope[*, 4]	3.70 + 0.61	2.98 + 0.44	3.52 + 0.53
Vanillin			
Threshold	1.6	0.08	0.3
Intensity intersect and slope[*, 5]	1.53 + 0.40	3.4 + 0.86	1.89 + 0.38

1. Data from Voirol and Daget, 1986.

* Magnitude estimations using 0.05% n-butanol as the standard. Regression correlations for log perceived intensity = *n* log concentration + K were all > 0.95

2. Rotameter-measured flow rate during exhalation was 40 ml/sec. The rate was stated to be lower than this for oral smelling, but no value was provided.

3. Rotameter-measured flow rate during orthonasal sniffing was 100 ml/sec.

4. The *n*-butanol standard concentration was matched by 40 p.p.m. citral for retronasal smelling; by 18 p.p.m., for orthonasal smelling.

5. The *n*-butanol standard concentration was calculated to require a concentration much above 8500 p.p.m. vanillin for intensity matching by retronasal smelling (8000 p.p.m. was the highest available); 4500 p.p.m. provided a match for orthonasal smelling.

during normal breathing.

Fully controlled studies of retronasal smelling would involve delivery to the oral cavity (or to the nasopharyngeal side of the olfactory mucosa) of specified air phase concentrations of known odorants. Although this degree of stimulus control is not uncommon in experiments involving only orthonasal olfaction (see Dalton, 2002), it has been rare for retronasal smelling (see Heilmann and Hummel, 2004, discussed in a subsequent section on 'Clinical Studies of Retronasal and Orthonasal Smelling', for a highly controlled comparison of retronasal and orthonasal smelling). In an early study, air phase citral or vanillin were presented to the oral cavity by Voirol and Daget (1986), who compared the resulting retronasal smelling thresholds and suprathreshold intensities with those for oral smelling (for the oral smelling condition, the odorants, in their mineral oil-alcohol solvent, were sipped) and for orthonasal sniffing. The two odorants are quite different: in air phase, vanillin is thought to have little or no trigeminal component, while citral is readily detected by anosmics (Doty, 1978). For both odorants, large threshold differences were reported, with the retronasal smelling threshold appreciably higher than the orthonasal threshold (Table 1). The difference was attributed to a much higher flow rate for orthonasal smelling. However, although it was stated that the oral smelling respiratory flow rate was the lowest of the three conditions, the oral smelling thresholds for citral and vanillin were much lower than the other two, suggesting that the supposedly tasteless citral and vanillin had major oral effects beyond those due to their air phase aspect. With regard to suprathreshold intensity, slopes for retronasal and orthonasal smelling were similar, although there was substantially greater intensity for orthonasal smelling. Once again, oral smelling did not resemble the other two (Table 1). Overall, these data suggested that for air phase stimulation, flow rate or access might be an important factor in the higher threshold and lower suprathreshold responsiveness of retronasal smelling. The results also suggested that stimulation by placing liquids in contact with the oral cavity can produce results that are very different from those based upon only air phase stimulation.

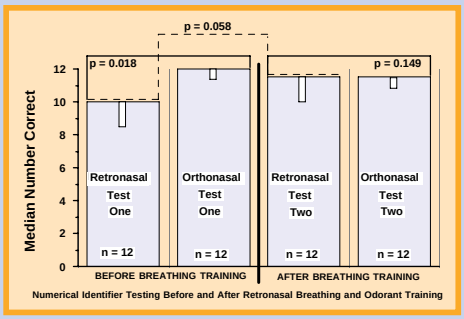

Figure 3. Median (and semi-interquartile range) number of correct retronasal and orthonasal odorant identifications after orthonasal odorant identification learning, before (1st) and after (2nd) retronasal breathing instruction and practice. Twelve (4 female) unpaid volunteer subjects, ages 18 to 24, screened for ability to identify by orthonasal smelling the veridical names of five common odorants but otherwise inexperienced in odorant perception experiments, learned to identify by orthonasal smelling without sniffing (only resting breathing was permitted) four other common odorants to which the numerical identifiers 1, 2, 3, or 4 had been assigned. Subjects were then tested for ability to correctly use these numerical identifiers with retronasal smelling (Retronasal Test One, far left hand column), and then with orthonasal smelling (Orthonasal Test One, 2nd from the left column). Next, subjects were instructed in a diaphramatic exhalation technique that was designed to increase the availability of retronasal odorants, and then retested with retronasal smelling using that breathing procedure (Retronasal Test Two) and finally with orthonasal smelling (Orthonasal Test Two, far right-hand column). Decimals above columns are Wilcoxon Signed Rank Test probability values (p), Bonferroni-corrected, for the difference between Orthonasal Test One and Retronasal Test One, between Retronasal Test One and Retronasal Test Two, and between Retronasal Test Two and Orthonasal Test Two. Data are from Pierce and Halpern (1996)'s experiment four and table IV.

About a decade later, Pierce and Halpern (1996), using air phase components of solid odorants that did not contact oral tissues when in the oral cavity (see Halpern, 2003), reported that learned retronasal smelling-dependent identifications of odorants were less accurate than identifications based upon orthonasal smelling unless breathing was modified such that tidal volume was increased (Figure 3). This outcome was consonant with the previous suggestion by Voirol and Daget (1986) that parameters of respiration were a factor in differences between retronasal and orthonasal smelling. Because only resting breathing and not sniffing was permitted for orthonasal smelling, respiratory flow rates (not measured by Pierce and Halpern) were probably less different than the factor of 2.5 observed in the Voirol and Daget experiment. The Pierce and Halpern (1996) study also tested the hypothesized (Rozin, 1982) total independence of the sensory systems involved in retronasal versus orthonasal smelling. This was evaluated by having the subjects learn arbitrary numerical identifiers for the odorants when smelled in one location, and then testing the extent of correct identifications when the odorants were presented in the other location. A lack of full independence was found. For example, correct identifications well above chance occurred with retronasal smelling when the learning had been with orthonasal smelling. Nonetheless, as already noted, correct retronasal identifications were fewer than correct orthonasal identification, unless modified breathing was employed during retronasal smelling (Figure 3). This outcome could indicate that retronasal and orthonasal processing of air phase odorants is identical, with differences in perception due only to lower air flow during normal exhalations. However, a significant limitation of the Pierce and Halpern (1996) study was the small number of easily learned odorant identifications, which could and did result in error-free identifications by some subjects. This ceiling effect (Ceiling and floor effects, 2004; Gent, 2004) precludes a conclusion that odorant identification abilities are the same for retronasal and orthonasal smelling.

A subsequent study using veridical name identifications of a larger numbers of odorants (all food-grade plant extracts), presented at maximum concentrations and smelled using only normal resting breathing,

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Retronasal and Orthonasal Smelling continued

Percent Identifications

ODORANT	Peanut	Coffee	Lemon	Cinnamon	Banana	Chocolate	Orange	Wintergreen
PEANUT	99.2	0.0	0.0	0.0	0.0	0.4	0.4	0.0
COFFEE	0.4	98.8	0.0	0.4	0.0	0.4	0.0	0.0
LEMON	0.0	0.0	97.5	0.0	0.0	0.4	2.1	0.0
CINNAMON	0.0	0.8	0.4	97.9	0.0	0.0	0.0	0.8
BANANA	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
CHOCOLATE	0.4	0.0	0.0	0.0	0.4	99.2	0.0	0.0
ORANGE	0.0	0.0	2.1	0.0	0.0	0.0	97.9	0.0
WINTERGREEN	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0

Figure 4. Percent retronasal smelling veridical-name identifications by 15 subjects of 8 randomly presented 100% concentration (neat) odorants. Data from Wininger and Halpern, 1999.

continued to encounter ceiling effects (Wininger & Halpern, 1999) (Figure 4). However, when appreciably lower concentrations (50% dilutions) were used with a different group of subjects, ceiling effects were eliminated for the retronasal smelling condition, and significantly inferior retronasal identification ability appeared (Figure 5) (Halpern et al., , 2000; Puttanniah & Halpern, 2001). Although these data confirm with food-related odorants that orthonasal identifications can be superior to retronasal, this outcome does not clarify the role of odorant access or flow rate differences in retronasal smelling, because only resting breathing was allowed, and flow rate was not measured. On the other hand, another observation, that the coffee odorant was not only identified correctly more often with orthonasal smelling but also that this odorant was consistently misidentified as oil when retronasal smelling was done (Figure 5), is intriguing. Almost perfect identification (97.5%) of this odorant mixture ('coffee and other natural flavors', with glycerin and water as the solvents [Frontier Natural Products Co-op, Norway, IA]) with orthonasal smelling, contrasted with misidentification as oil on 27.5% of the retronasal trials (Figure 5), may imply that processing of retronasal smelling is divergent from that for orthonasal smelling, irrespective of any differences in flow rate.

More recent studies from my laboratory have reported that pairing an orthonasal odorant with a different retronasal odorant during natural breathing cycles that alternate orthonasal and retronasal smelling can produce an asymmetric decrease in the correct identifications of the orthonasal odorants (Sun & Halpern, 2001, 2002). This surprising outcome may support the hypothesis that retronasal and orthonasal smelling are processed differently.

Studies of retronasal smelling that employed food-grade liquid extracts as odorant sources, placing these sources in intraoral containers (e.g., Halpern et al., , 2000; Halpern, 2002; Puttanniah & Halpern, 2001; Sun & Halpern, 2001, 2002; Wininger & Halpern, 1999), have assumed that food-associated mixtures are especially relevant for retronasal smelling (Wilkes et al., 2003), and that natural resting breathing provides a flow rate, volume, and temporal pattern that is appropriate for this sensory system. While this may be correct, an important limitation is the unknown air phase concentration within the oral cavity. In addition, although the odorant presentation containers do prevent contact between the odorant sources and oral tissues, they are necessarily a source of sustained, albeit tonic, mechanical stimulation of the tongue and teeth. An alternative approach, utilized by Voirol and Daget (1986), directly delivered air phase odorants to the oral cavity. This permitted concentration to be known and still allowed either single pure odorants or defined mixtures. Homma et al. (2003) combined direct delivery of odorants to the oral cavity with the technique of gas chromatography olfactometry (Acree, 1997; Deibler et al., 1998), which permits components of mixtures to be sequentially judged for intensity or quality and simultaneously characterized by gas chromatography. Using this technique, Homma et al. (2003) reported that, in general, retronasal smelling was less sensitive than orthonasal smelling, and less effective in describing odorant quality. However, they found this to not be the case for some components of mixtures, and also observed that for some odorants, retronasal smelling descriptions differed from orthonasal descriptions. The qualitative aspect of these outcomes are reminiscent of those previously observed using food-grade extracts in odorant presentation containers (Halpern et al., 2000; Puttanniah & Halpern, 2001).

CLINICAL ASPECTS OF RETRONASAL AND ORAL SMELLING

Retronasal or oral smelling have been addressed in several clinically-related studies. Oral smelling of a set of powdered foods and flavors has been proposed as an appropriate method to assess retronasal function, and as a useful means for comparing retronasal and orthonasal competence (Heilmann, et al., 2002). The authors suggested that retronasal defects only occur when problems with orthonasal smelling are observed. In another study (Duffy et al., 2003), oral smelling intensity judgments of a sweetness and odorant source (jellybeans) were found to be correlated with judged taste intensity of the same source, with several tests of orthonasal olfaction, as well as with taster status, number of fungiform papillae, and judged taste intensity of quinine hydrochloride applied to the tip of the tongue. In contrast, orthonasal olfaction was correlated only with orthonasal smelling intensity. These data led the authors to suggest that damage to the chorda tympani (CT) nerve, which innervates the taste buds of the anterior portion of the tongue (Halpern, 2002), was related to reduced oral smelling sensitivity. This could mean that if an individual's CT is damaged, oral smelling, and hence flavor, will be compromised. At a more general level, the data indicate that oral smelling has a major gustatory component.

Quite different outcomes can be found when retronasal smelling is measured. Investigations employing odorant presentation containers that prevented direct contact between odorants and oral tissues (Halpern, 2003) found that defects in retronasal smelling can occur in the absence of orthonasal deficits (Cowart, et al., 1999, 2003). These observations of compromised retronasal smelling without problems in orthonasal smelling emphasize the distinction between oral smelling and retronasal smelling. Evaluation of oral smelling can be an important clinical goal (Cerf-Ducastel and Murphy, 2001, 2003, 2004; Heilmann, et al., 2002; Lèger et al., 2003), because the measure captures responses to air phase, gustatory, and chemesthetic input. However, if comparisons to orthonasal smelling are intended, it may be necessary to characterize retronasal smelling.

Event-related potentials (ERP) can be a useful clinical diagnostic tool (Dalton, 2002; Kobal and Hummel, 1991; Kobal, 2003). In a very sophisticated study, Heilmann and Hummel (2004) measured not only odorant EPR (OERP) to orthonasal and retronasal smelling, but

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Retronasal and Orthonasal Smelling continued

A. Retronasal Smelling Percent Identifications

ODORANTS	Coffee	Lemon	Cinnamon	Banana	Orange	Strawberry	Wintergreen	Oil (canola)
coffee	47.5	7.5	2.5	7.5	0.0	5.0	2.5	27.5
lemon	2.5	42.5	2.5	7.5	22.5	7.5	0.0	15.0
cinnamon	5.0	5.0	70.0	0.0	5.0	0.0	10.0	5.0
banana	0.0	5.0	0.0	75	2.5	2.5	5.0	10.0
orange	0.0	22.5	0.0	5.0	62.5	0.0	5.0	5.0
strawberry	0.0	2.5	2.5	15.0	0.0	75	2.5	2.5
wintergreen	0.0	2.5	2.5	5.0	7.5	0.0	77.5	5.0
Oil (canola)	7.5	12.5	2.5	7.5	5.0	2.5	5.0	57.5

B. Orthonasal Smelling Percent Identifications

ODORANTS	Coffee	Lemon	Cinnamon	Banana	Orange	Strawberry	Wintergreen	Oil (canola)
coffee	97.5	0.0	0.0	2.5	0.0	0.0	0.0	0.0
lemon	0.0	60.0	0.0	0.0	27.5	0.0	10.0	2.5
cinnamon	0.0	0.0	95.0	0.0	0.0	0.0	2.5	2.5
banana	0.0	0.0	0.0	90	2.5	5.0	0.0	2.5
orange	0.0	32.5	0.0	0.0	67.5	0.0	0.0	0.0
strawberry	2.5	5.0	2.5	2.5	0.0	85	2.5	0.0
wintergreen	0.0	0.0	2.5	0.0	0.0	0.0	97.5	0.0
Oil (canola)	0.0	7.5	5.0	5.0	10.0	0.0	0.0	72.5

Figure 5. Percent veridical-name identifications by 20 subjects (9 female, ages 18 to 31), not selected for ability to smell, of 8 randomly presented 50% diluted food-grade extracts of plant materials. Subjects learned identifications during orthonasal smelling, and then were tested using retronasal smelling (A) and then orthonasal smelling (B). Cells with gray backgrounds are percentages for correct identifications. Numbers of retronasal errors were significantly greater than orthonasal errors ($t = 3.518$, $p = 0.0012$). Correct identifications for the coffee odorant were significantly greater for orthonasal smelling ($t = 5.63$, $p = 0.0063$), and incorrect identification of coffee as oil were significantly greater for retronasal smelling ($t = -4.8$, $p = 0.0062$). All p-values were Bonferroni corrected. Data from Halpern, Puttanniah, & Ujihara, 2000 and Puttanniah & Halpern, 2001.

also psychophysical thresholds and suprathreshold intensity judgments . The subjects all had normal physical nasal structures and were found to be normosmic for orthonasal and retronasal smelling before testing began. Odorants were delivered from an air-dilution olfactometer through tubes directly into the nasal cavity. For orthonasal smelling, a delivery tube ended just past the nasal valve, about 1.5 cm beyond the anterior naris; for retronasal smelling, in the epipharynx (i.e., nasopharynx), about 7.5 cm beyond the anterior naris (nostril) (Figure 1). Delivered odorant concentration was calibrated before judgments or OERP measurements using a third tube, ending in the olfactory cleft, that was connected to an 8.3 Hz sampling rate (faster than the breathing cycle) 'electronic nose', receiving 200 ml/second samples. For the odorant hydrogen sulfide (H_2S), retronasal and orthonasal calibration curves overlapped. Threshold judgments were measured using staircase procedures for 12 subjects (5 women, mean age 27, age range 23-33) for two odorants: a chocolate flavoring and lavender. For both odorants, retronasal smelling threshold was significantly higher than orthonasal, thus confirming the earlier report by Voirol and Daget (1986). However, in distinction to previous studies, the highly controlled conditions of the Heilmann and Hummel (2004) study made it unlikely that odorant access or flow rate differences could account for the disparate thresholds. An elevated retronasal smelling threshold for the chocolate odorant is important because food-related odorants have been suggested to be especially relevant for the retronasal location. In the second part of the experiment, intensity judgments and OERP for H_2S and phenyl ethyl alcohol (PEA) were measured for 20 subjects (10 women, mean age = 25.6, range = 21-36 years), after subjects were trained in velopharyngeal closure (see Halpern, 2003) in order to isolate the nasal cavity from the oral cavity by elevating the velum (Figure 1). Judgments were made with a joystick and a computer-displayed horizontal line. It was found that retronasal intensity was significantly lower for H_2S but not for PEA. The absence of an intensity difference for PEA is interesting because air-phase PEA is generally thought to provide minimal or no trigeminal stimulation (Dalton, 2002; Doty et al., 1978); in contrast, H_2S is considered to be both an olfactory and a trigeminal odorant. Consequently, this outcome may indicate that judged intensity differences for retronasal versus orthonasal stimulation are likely to involve both olfactory and trigeminal components, perhaps with a greater trigeminal role for orthonasal smelling. In contrast, for the OERP measurements, differences for PEA stimulation were observed for both P2 amplitude and N1 latency (there were no differences for N1 amplitude) but for H_2S no retronasal versus orthonasal stimulation differences were found. One might have expected larger OERP responses to the H_2S stimulation.

SUMMARY

Orthonasal implies correct; retronasal, backward. The range of environmental events that may provide orthonasal stimulation is much wider than that for retronasal inputs, but retronasal events are a rich and powerful factor in judgments of flavor. Because odorant stimulation in humans generally reaches both olfactory and trigeminal mucosa, the terms retronasal and orthonasal smelling are used here rather than orthonasal and retronasal olfaction. Both are distinguished herein from oral smelling, i.e., the circumstance in which liquids or solids are delivered to the oral cavity with the intention that they will serve primarily as retronasal odorants, but without consistent evidence for a concomitant lack of gustatory or chemesthetic stimulation. Retronasal smelling lacks the perceptual and multimodal richness, and ecological validity, of oral smelling, but affords direct comparisons to orthonasal smelling.

Empirical data demonstrated that thresholds for retronasal smelling were higher than those for orthonasal smelling, with odorant access unlikely to be the critical factor. This implies differential processing, perhaps dependent upon different spatial-temporal patterns. Retronasal ability to identify odorants was inferior to orthonasal for a range of odorants, but the underlying basis is unknown. Judged intensity differed between retronasal and orthonasal smelling for some odorants but not for others, as did odorant-based event-related potentials, with access removed as a reason but trigeminal versus olfactory stimulation possibly relevant. Some clinical studies suggested the possibility of impaired retronasal smelling accompanied with normosmic orthonasal smelling, and both fMRI and PET measurements found differences between orthonasal and oral smelling.

ACKNOWLEDGEMENTS

Prepared during support from Hatch-CSREES-CUAES NYC-191403. Shreya Sood provided comments on an earlier draft of the manuscript.

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Retronasal and Orthonasal Smelling continued

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NEWS

Pangborn 2005 Announced

The first announcement and call for papers for the Sixth Pangborn Sensory Science Symposium has been made. It will be held from 7-11 August 2005 at the Harrogate International Centre, UK. The abstract deadline is 31 January 2005. For information visit www.pangborn2005.com ■

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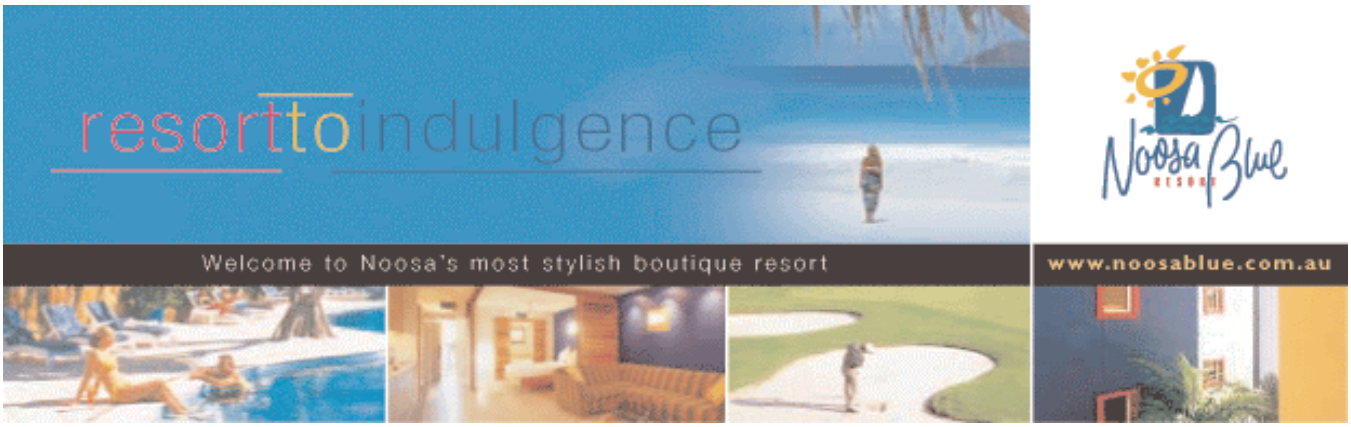

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NEWS

Vale: Rainer Voight (1951-2004)

All through his career, Rainer Voigt has been an active member of the international Chemoreception community. For his PhD he worked on olfaction in goldfish behaviour with Hanns-Peter Zippel in Goettingen. With his postdoc in Woods Hole with Jelle Atema, he switched to lobsters where he quickly helped to establish the lobster as a olfactory neurophysiological preparation and model. He was an expert in extracellular nerve recordings and could extract more information from a single fiber than just about anyone, while remaining interested in behaviour. After a 2-year interlude working on fish lateral line with John Montgomery in Auckland he returned to Atema's lab briefly before moving in 2002 to Paul Moore's lab at Bowling Green State University in Ohio. There he worked primarily on crayfish chemoreception.

Although he has published over 40 papers on various aspects of chemoreception and behaviour, his greatest legacy will be his tireless effort in training young students. He showed infinite patience teaching the techniques of recording from nerves and whole animals and educating them in rigorous scientific investigation. Typically, students were initially a bit scared of his serious "German demeanour" until they discovered the warmth of his heart and his dedication to them and their science. The many who got to know Rainer over the years will remember his fondness and his dedication to great science. In addition to the training of students, Rainer was always helpful in collaborating with senior scientists. He impacted the research careers of many people.

Rainer passed away after a battle with liver disease and is survived by his mother, Ingrid Voigt.

At AChemS 2004, a group of Rainer's friends decided to establish a memorial fund for the support of graduate students at the Boston University Marine Program. Rainer's impact on students was always impressive and supporting students was one of Rainer's greatest pleasures in life. If you would like to make a contribution to the "Rainer Voigt fund for graduate student support", send to:

Voigt Fund
Boston University Marine Program
Marine Biological Laboratory
Woods Hole, MA 02543, USA.

Paul Moore and Jelle Atema

WineSense:

Sealing and storing bottled wine: assessing the sensory properties of wines stored under corks, screw-caps and plastic stoppers

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The Australian wine industry, and indeed the global industry, has in recent years increasingly looked to alternatives to natural cork bark for sealing bottled wine. Stoppers or 'closures' made from cork tree (*Quercus suber*) bark have been used for sealing bottles of all types for hundreds of years, with wine being one of the last products to continue to use natural cork as a seal.

Natural cork does not always meet winemakers' or consumers' requirements for a seal. Some corks can leak, which is clearly undesirable and easily assessed. A more subtle problem with natural cork closures is they can in some circumstances allow the wine to oxidise over time, resulting in unpleasant, aldehydic flavours as a result of slow oxygen permeation through the material. They can also confer taints to a proportion of bottled wine, notably a musty/mouldy off-flavour due to trace amounts of the potent volatile compound 2,4,6 trichloroanisole (TCA).

The continued use of cork as a closure is partly due to a desire for some premium quality, often high priced red wines to be stored for periods of years for improvements in flavour, together with the largely unsubstantiated belief by some that red wine matures best with the slow permeation of small amounts of oxygen through the seal. The supposition is that natural corks allow such permeation. Although only a small percentage of wines might be aged for extended periods before consumption, there is almost certainly a perception in many markets that if high quality wines require corks, lower priced wines should also have them. This is notwithstanding the fact that many ultra-premium wine bottles may be re-corked after extended storage due to degradation of the cork, and the widespread knowledge among wine enthusiasts that opening an old bottle of wine owes a good deal to chance, with some individual bottles having aged well, and others having become oxidised. In addition, the amount of oxygen, if any, required for optimal maturation remains an area of active research.

Alternatives to natural cork include screw cap closures, so-called 'technical' corks -corkwood-based closures that also contain a synthetic component- and synthetic closures manufactured from polymer materials. These alternative closures have become more widely available and have in recent years started to be used by wine producers worldwide.

With a range of closure types available, wine producers require information to allow an appropriate choice to be made as to the suitability of particular closures for their products. Formal sensory descriptive analysis profiling studies have been carried out with several closure studies in recent years at the AWRI. A large study investigating a white wine bottled using 14 different closures, including a screw cap, natural corks, technical corks and synthetic closures, has had a major sensory analysis component, in addition to numerous chemical and physical measures (Godden et al 2001, Francis et al 2003). Analyses, including sensory, have been carried out from six months post bottling to four years post-bottling. This has been a collaborative project involving AWRI specialists in several areas, under the overall project leadership of Peter Godden, Winemaker and Manager - Industry Services. The sensory measurements have been made using a panel of ten experienced tasters at each testing time. Assessments were carried out under controlled conditions using the AWRI sensory facilities, taking into account conventional good sensory practice procedures, with independent assessments of randomly ordered samples presented monadically in coded, standard ISO wine tasting glasses. Since the 12-month period, at each testing time eight replicate bottles of each of the tested closures were assessed. The assessors rated the intensity of a number of attributes using a 0-9 category scale, with aroma and 'in-mouth' attributes assessed independently.

There were found to be large differences among the closures from the earliest testing period. One of the tested synthetic closures conferred a strong styrene-like taint and wine stored under this closure was rated as highly oxidised only six months after bottling. A number of the synthetic closures were rated as significantly less fruity (attributes such as citrus, lime and pineapple) from a relatively early stage after bottling and were rated progressively higher in oxidised character over time. The screw cap sealed wine retained the highest scores for fruity flavour attributes. The other major attribute that differentiated the samples was mouldy/musty aroma due to TCA, which was an attribute that was rated highly for individual bottles of all of the cork-based closures. A relatively

cont. pg 13

WineSense continued

subtle 'struck flint' or rubbery attribute was also perceived in the samples. At the 36 months testing time, the wine bottled with the screw cap was highest in this attribute, but other closure samples were also relatively high in this character, and this attribute was positively correlated with the fruity attribute ratings given to the wine. Some closures also conferred a slight plastic or glue-like flavour but this was not evident for the majority of the closures examined.

The major sensory differences among the closures were correlated with chemical measures found for the wines. The study showed that relatively simple chemical measures made quite soon after bottling (for example, sulfur dioxide and spectrophotometric absorbance values) could be used to predict most of the sensory ratings at two or three years post-bottling

Following the success of the initial large study, which is in fact still on-going, additional, somewhat smaller, studies are being carried out. One research study has investigated a red wine bottled with a screw cap with different levels of air present in the headspace above the wine at bottling, in comparison to natural cork and a synthetic closure. A separate research project has investigated the effect of the presence of ascorbic acid, which is used as an antioxidant in some white wines, for a small range of closure types, including two natural corks, a synthetic closure, and a screw cap. The AWRI Analytical Service is also carrying out investigations on a commercial contract basis, allowing suppliers or manufacturers to have their closures assessed in comparison to a number of reference closures. All of these studies have involved the generation of systematic sensory data repeated over time, together with compositional data, and the results will be published in due course.

As a result of research studies carried out into the performance of closures, the wine industry and closure manufacturers have been able to make decisions as to the appropriateness of particular sealing technologies for different wine styles and for wines destined for different periods of cellar storage. Over the last few years, there has been a resurgence in the use of screw cap closures for bottled wines, particularly for fruity white wines but also for some premium red wines, in Australia and New Zealand - as well as other parts of the world - and this has likely been partly influenced by the information gained by the industry from the AWRI studies.

Acknowledgements

This work is financially supported by Australia's grapegrowers and winemakers through their investment body the Grape and Wine Research and Development Corporation, with matching funds from the Australian Government. The contribution of many AWRI colleagues, but in particular Peter Godden, Elizabeth Waters, George Skouroumounis, Kate Lattey, Mark Gishen, Adrian Coulter, Ella Robinson, Mariola Kwiatkowski, Patrik Jones, Sakkie Pretorius and Peter Høj in the work outlined is gratefully acknowledged.

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E-Noses to Silence Smell Complaints

Continuous real-time monitoring heralds a generational shift in pollution control

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Why do some industries make such obnoxious odours? What is this costing them and us? Do they know what they are doing? What can be done about it? These are four of many questions on the issue of air quality in practically all city and country towns in Australia and elsewhere.

What many industries do, is of necessity, a smelly or chemically noxious business. Their livelihoods depend on the use of volatile chemicals or they produce them as by-products or waste. They often establish their plants and facilities a long way out of town, but, with time and "progress", the suburbs eventually reach them, and that is usually when complaints begin.

Individual complainants, however, are no longer the only cause for concern by the industries. Growing community awareness, of the wider effects of pollution and resource usage, are bring pressures to bear on an increasing number of industries, including those that are remote from urban communities.

Complaints very often concern smells from organic activities, such as farming and food processing. Among the most "celebrated' are the smells from animal product processing, and from raising of animals in confined spaces: such as cattle feedlots, piggeries and chicken sheds. Among the top stink-makers are sewerage and waste treatment plants. Many other industries release chemicals that are not quite as unpleasant, but may be of far greater concern. Examples of those, which release solvents into the atmosphere: from the small vehicle spray painter to large plants, such as metal smelters or oil refineries. Closer to home there is the motor car or small business truck contributing to the reduced quality and safety of the air we breathe.

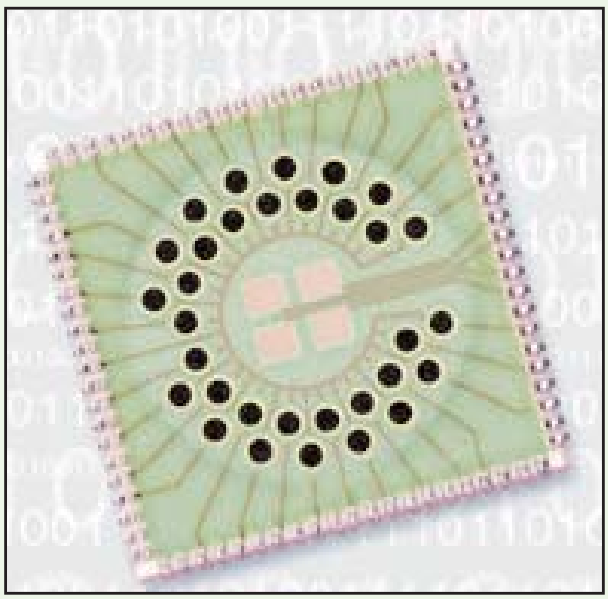
The costs include the individual's health and discomfort, and the time taken to make and process the complaint. Affected industries acknowledge that complaints cost millions every year. They have to devote valuable people and resources to investigating the issues raised, documenting and recording their responses, dealing with possible and real litigation, and complying with orders from authorities such as an environmental protection agency (EPA). These events can result in costly action and unplanned expenditures.

At the national level the cost adds up to billions of dollars. Part of this is the price an industrialised society must now pay for operating in a clean and healthy environment and sustaining its long-term future. The rest, probably the greater proportion, is arguably a waste of resources that produces nothing. It is this fraction that can be reduced by better use of technology, and the savings redeployed to other ends.

Industrialists know they have a problem, but only in general. Specifically, each situation is made complex by the intermittent nature of emissions; variations in their quality and intensity; and the vagaries of external factors such as temperature, humidity, wind speed and direction. People at the plant adapt to the smell, and for them personally, the problem simply doesn't exist. By the time a smell is investigated the situation has usually changed: usually it has abated or changed in quality.

The current methods of establishing the strength, quality and source of the environmental odour are very difficult to carry out rapidly: they consist of drawing air into clean non-adsorbent plastic bags, from various parts of the site or downwind from it. The air is then taken, within 30 h, to a lab where chemical analysis is performed or a panel of humans assess it.

The trained human panel of 6 to 8 people sniff systematically diluted samples of the original air sample, to determine how many times a unit volume of that air has to be diluted before it can no longer be detected. These numbers are called "odour units" (OUs). Each bag sampled at any one time produces a single odour unit number. The higher the number the more strongly smelling the air is assumed to be. The effort and cost required to obtain these numbers means that not many numbers can be obtained, either over time or over a wide area, and that rapid response



A Cyranose chip with 32 proprietary composite polymer sensors. From: Skelley, (2000) with acknowledgment to Cyrano Sciences, USA.

E-Noses to Silence Smell Complaints continued

to a complaint or timely action to minimise a possible complaint is practically impossible.

What can be done?

What is needed is a continuous monitoring technology with sufficient sensitivity, reliability (reproducible results) and validity (measuring what it claims to measure). Srivastava and Levy (2002) defined the optimal characteristics for an air quality monitoring system within constraints imposed by each situation's factors, such as likely concentration range of pollutants; background emissions from other sources; meteorological and geographic conditions; and measurement and calibration frequency of the system. The criteria are, briefly:

1. Sensitivity. It must meet the concentration ranges required for the job and be able to discern one quality of odour from another
2. Reliability. It must consistently produce accurate, precise, specific and reproducible results
3. Temporal Resolution. It must have a sufficiently short time period over which it makes a determination
4. Robustness. It must have a low failure or fault rate and its performance must be steadfast against such destructive influences as extremes of temperature, dust, wind movement and humidity (including rain or snow).

In addition, a monitoring system should be affordable, of convenient size, draw minimal power and communicate efficiently with its operator.The E-Nose solution

Inspired by biological systems, a technology is emerging from the pure science of the olfactory system (see review of possibilities in Bell, 1996). Novel surfaces which capture different species of molecule have been combined into arrays that mimic the biological nose to produce a unique, albeit fuzzy, representation of a complex mixture of volatiles. Various chemical sensor arrays (electronic or e-noses) have been successful, to varying degrees, at meeting the above criteria for detection and monitoring of airborne chemicals.

A growing number of labs and companies have become involved in recent years. A cursory web search on www.google.com for the words "electronic nose" will yield at least 143,000 references. There are currently between 20 and 30 labs and companies working on e-noses. Interest in technical and intellectually challenging problems associated with e-noses is manifested in the lively web user groups and a growing number of international conferences.

Companies producing e-noses for commercial applications include Cyrano Sciences (Pasadena, CA) who have targeted medical diagnostic applications and screening of food and packaging quality, with a hand-held device called "Cyranose". Marconi Technologies (Chelmsford, Essex, UK) has used e-noses to detect gas leaks, to monitor the quality of propylene glycol in lotions, and freshness in frozen shrimp. AromaScan, (Crewe, Cheshire, UK) has placed over 200 electronic noses into laboratories around the world, including one reportedly installed on the Mir space station to detect odours from failing electronic components (Skelley, 2000).

A new generation of electronic chemical monitoring devices (e-noses) promises to change this situation for the good of all concerned. The arrays are usually smaller (fewer sensors) than that shown in the illustration and target specific problem odours. The kind of odour, its context and its chemical composition are studied first, and then an array is specified, that best responds to the priority odour. The specialised e-nose responds at levels that give rise to complaints, against and discernible from characteristic background odours.

These e-noses can be readily combined as multi-unit networks to provide wide area or very long perimeter monitoring of a large installation or zone of interest. Outdoor security and military applications are areas of potential application in which these new generation sensors are likely to appear in the near future.

Smaller yet bigger?

The development of the internet, geo-positioning and communications by satellite makes the retrieval of information possible from multiple e-noses deployed extremely remotely. The economics of scale possible in the electronics industry dictates that cost-per-unit will become trivial and thereby allow networks of limited-life, self-disposable e-noses to be deployed by the thousand. Operating when visual and vibrational detectors may be ineffective, these new electronic noses may play a vital part in surveillance along a country's border; in difficult-to-reach mountainous areas; or even across expanses of ocean.

The amount of odour data that can be gathered over an expanse of terrain makes mapping of flow dynamics from industrial sites into areas of complaint or concern possible at a level of accuracy only ever dreamed of by pollution modellers.

E-Nose Downunder

The Australian company, E-Nose Pty Ltd, a spin-off of UNSW (see Hibbert and Barnett, 2002), now has a range of relatively small, specialised e-nose "sentinels" that are tailored for specific odour environments such as abattoirs and sewerage treatment plants and pumping stations. The company has also developed a novel self-diagnostic and calibration system to add-on, when appropriate, to each e-nose. The combinations of sensors is selected to do the job needed by the client, with resulting robust and reliable operations at levels of sensitivity well within the range of reported nuisance odours.

The systems were trialed in industrial settings, in "hostile" (temperature, humidity and particulates) indoor and outdoor settings, and proved to be very robust, meeting adequately all four criteria listed by Srivastava and Levy (2002).

The system was calibrated against human "odour unit" measurements and provided useful data down to 1 OU. The speed at which this human-calibrated data can be gathered and analysed heralds a *generational shift* in odour measurement and monitoring.

It makes possible realistic mapping of the dispersion of odours from industrial sites at different times and in different settings, thereby bringing clarity to these issues. This will be a "win-win" for the industrialists and the community.

Remote access by internet allowed the operator (and the central development lab) to observe the dynamic status of the odours emitted by his plant at a rate of more than one measurement per second. They could observe at any time, the current and past performance of the plant over the past days and weeks.

In addition, the company developed software to predict the likelihood that the plant will exceed community complaint levels in the coming 30 minutes. This was achieved with a reliability coefficient of 0.96.

The savings in costs to the industries, once the technology is adopted, will be massive. They will be constantly equipped with information, and will manage their odour abatement investments more efficiently. Freed from constant threat of litigation from the community and EPAs, they will be able to get on and grow their businesses without relocating and will have newfound resources to apply to better things.

Addendum

E-Nose Pty Ltd would like to hear from anyone interested in assisting it in commercialising its inventions by installing prototype systems into their industrial settings (contact g.bell@atp.com.au). The products are scheduled to be launched commercially early in 2005.

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Upcoming Events

5 - 9 July, 2004	**XIII International Symposium on Olfaction and Taste (ISOT) / JASTS** Kyoto, Japan Abstracts Deadline: 10 March Registration Deadline: 14 April http://epn.hal.kagoshima-u.ac.jp/ISOT2004/
24-29 July 2004	**12th Australian Wine Technical Conference** Melbourne, Australia http://www.awitc.com.au
25-28 July, 2004	**37th Annual AIFST Convention** Brisbane, Australia Contact: aifst@aifst.asn.au or www.aifst.asn.au
28-30 July 2004	**7th Sensometrics Meeting** Davis, California USA Info: http://www.statistik.uni-dortmund.de/sensometrics/
1-3 October 2004	**Australasian Association for ChemoSensory Science (AACSS)** 7th Annual Meeting Noosa, Queensland, Australia http://get-me.to/aacss
21-24 June 2005	**11th Weurman Flavour Research Symposium** Comwell Roskilde, Denmark Info: weurman2005@staff.kvl.dk www.weurman2005.kvl.dk
2005 (Q3/4, Month TBC)	**AACSS on Heron Island** (Australian Great Barrier Reef) Start planning to come! Info: g.bell@atp.com.au

ChemoSense (ISSN 1442-9098)
Web: http://www.chemosensory.com
Published by **E-Nose Pty Ltd**
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Design and Layout: Lawton Design Pty Ltd

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