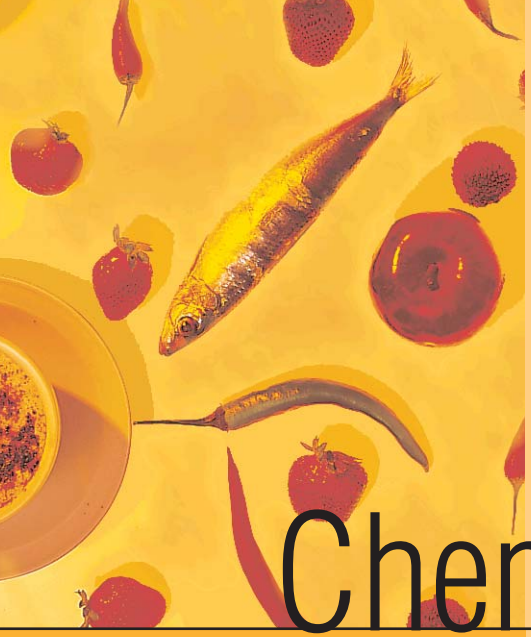
Vol. 6 No.1 December 2003



ChemoSense

EDITORIAL

Life without Olfaction Reveals Life with Olfaction

By Graham Bell

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Research and clinical practice shows that smell and taste play a significant part in our lives. Two short reviews written for this issue of *ChemoSense*, by leading scientists and clinicians in the USA, show how loss of our chemical senses impact on our safety, health and quality of life. We need to value olfaction more than we do. Individuals who are approaching 60 yrs can prepare for life with diminished olfactory acuity: for increased risks, disrupted diets and broad impacts on their health, including weight loss from reduced palatability of food. By contrast we learn how much olfaction really matters in normal healthy life. In fact, every day, our olfactory sense, of which we are mostly unconscious, is keeping us healthy, safe, competent and happy.

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This is our 21st issue, since *ChemoSense* was first published in November 1998. The Editor and Staff wish our readers, advertisers and authors a Happy Holiday and a Peaceful, Prosperous 2004 ■

The Overlooked Impact of Olfactory Loss: Safety, Quality of Life and Disability Issues

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Introduction

Can you imagine a life without your sense of smell? Memories of awakening to the smell of a hot breakfast permeating the house would not exist. Vibrant, fresh-cut spring flowers would have the olfactory appeal of brown autumn leaves. One would never know that the last glass of milk in the refrigerator was spoiled until the first nauseating gulp filled the mouth. The special scents of loved ones would no longer be there to remain with you even after that person had left your side. And tragically, your first warning of a fire might be the heat of the flames on your skin, or the even the terrified screams of your family members already threatened by the blaze.

There are many ways that the sense of smell enhances, and even protects our lives on a daily basis. However, for those of us who continue to enjoy intact olfaction, this is easily taken for granted. Similarly, it is hard to comprehend the many ways that one's life could be impacted after suffering a loss of the sense of smell. In this review we discuss loss of olfactory function and its impact on disability, safety, health, quality of life.

Over the past two decades, researchers have made tremendous strides in our understanding of the physiological basis of olfaction. Modern molecular

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ISSN 1442-9098

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biological techniques have elucidated the genetic basis of olfactory receptor specificity, while electrophysiological methods have advanced our understanding of the neural encoding of olfactory stimuli. Unfortunately such landmark basic science advances have not translated into increased awareness of olfactory disorders, their treatment and sequelae within either the medical or the lay communities. However, olfactory deficits can have significant effects on many aspects of our day-to-day lives. Some of these effects may seem trivial in themselves, but when all are considered together, it is clear that olfactory dysfunction can seriously impair a person in their day-to-day activities and occupation, increase their risk of injury or even death, and reduce their overall quality of life.

The limited attention given to olfactory disorders likely results from a variety of factors. First, both health care providers and the lay public tend to underestimate the rather high prevalence of olfactory dysfunction. There are numerous causes of olfactory dysfunction, including aging, medications, viral infection, paranasal sinus disease, head trauma, and iatrogenic injury. Virtually all age groups may be considered at risk for developing olfactory dysfunction. Estimation of the true prevalence of olfactory dysfunction is however difficult. There is no uniform mechanism for reporting, such as exists for some infectious diseases and cancers. In addition, given the rather poor understanding of olfactory disorders among the lay public, many with olfactory dysfunction may not even think to seek help, while those that do may not know where to turn for assistance. A significant percent of cases may thus go undetected. The estimated prevalence of olfactory disorders in the United States, based upon the National Health Interview Survey administered through the National Institutes of Health, was 2.7 million[1]. Murphy et al estimated that olfactory impairment may affect as many as 14 million Americans of age greater than 55 years[2]. Given the increasing incidence of olfactory dysfunction with aging, the overall prevalence will likely rise in coming years as life expectancies continue to increase, and the proportion of elderly in the population grows.

Second, despite advances in our understanding of the physiological basis of olfaction and its disorders, treatment options available to medical practitioners remain limited. In general, conductive olfactory disorders, resulting from impairment of odorant access to the olfactory neuroepithelium, may be treatable. For example, a patient with severe sinonasal polyposis may experience improvement in olfaction following medical or surgical management of the polyposis, with reestablishment of nasal airflow. However, the vast majority of patients with neurosensory deficits, in which the deficit lies within the olfactory neuroepithelium or central olfactory pathways, typically have no other treatment available than time and possible spontaneous recovery. This may create a sense of frustration within the medical community, and curb physicians' interest in the evaluation and management of patients with olfactory disturbances. This is evidenced by the fact that, despite the number of patients with olfactory disturbances, there are still relatively few smell and taste research centers worldwide.

Lastly, there is relatively little information available to the medical community, and even less available to the lay public, detailing the negative impact of olfactory dysfunction on people's lives. In the following discussion we will review some of the literature available on this subject.

Disability

Olfactory impairment has historically been overlooked as a public health problem. In contrast to deficits in other senses such as sight or hearing which are clearly considered medical conditions with considerable associated disability, most tend to relegate olfactory dysfunction to the status of mere annoyance rather than medical disability. The American Medical Association *Guides to the Evaluation of Permanent Impairment* states: "Only rarely does complete loss of the closely related senses of olfaction and taste seriously affect an individual's performance of the usual activities of daily living. For this reason, a value of 1 % to 5 % impairment of the whole person is suggested for use in cases involving partial or complete bilateral loss of either sense due to peripheral lesions..."[3] In contrast, loss of vision carries a level of impairment up to 85% for complete bilateral blindness, with varying degrees of impairment assigned to partial or complete limitations of either acuity or visual fields. Loss of hearing is assigned a level of impairment up to 35 % for bilateral complete loss of hearing, while a monaural complete loss is assigned a level of impairment of 6 %. Although people in general are far more dependent on their vision or hearing in their day-to-day life, olfaction clearly serves several important, and potentially even life-saving roles in day-to-day life.

> "One of the things that attracted me to my husband was his wonderful smell. Each of our children had a distinct, sweet smell. These former pleasures of life are gone." -
>
> 63 year old female bed and breakfast owner with severely impaired olfaction after motor vehicle accident

Safety and Survival Functions

Chemical senses have important survival functions in virtually all living organisms. Many animals rely on their olfactory function for their survival. Deer depend on their keen sense of smell to identify predators hundreds of yards away, thus allowing them to flee in safety. Sharks' ability to detect even a drop of blood in ocean water has helped these predators to survive for millions of years. Innumerable other examples exist of animals dependent on their sense of smell to locate food sources, avoid predators, and identify mating partners or their young.

While the survival role of human olfaction may not be quite as obvious, it cannot be overlooked. An

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intact sense of olfaction is critical to the early detection of a variety of health hazards. In a survey of patients who had previously been evaluated for olfactory loss at two major smell and taste research centers, 75 % of patients reported impaired detection of spoiled foods, 61 % reported impaired detection of gas leaks, and 50 % reported impaired detection of smoke[4]. In another study, 50 % of patients with olfactory deficits reported ingestion of spoiled foods, and 30 % having burned food[5]. In a review of the demographics of fire casualties, Hall found that the elderly are involved in a disproportionate number of both house fires and gas poisonings[6]. Given the well-documented correlation with increasing age, olfactory dysfunction may be a factor in the increased risk of fire-related deaths in this population. Obviously other factors may also apply, and further research is needed to conclusively identify a contributory role of olfactory dysfunction. An investigation to better quantify the risk of experiencing hazardous events such as fire, gas leak, or toxic ingestion in patients with olfactory impairment is currently underway at the Smell and Taste Clinic of Virginia Commonwealth University.

Health Impact

Chemosensory deficits may not only reduce patients' enjoyment of foods, but may also place them at risk for long term nutritional or health sequelae. Duffy et al studied 80 high-functioning elderly women, who were evaluated for olfactory disturbances and nutritional risks[7]. They found that subjects with lower olfactory function on testing had lower interest in food-related activities such as cooking or eating a wide variety of foods, lower preference for foods with predominant sour or bitter taste, higher intake of sweets, and a nutrient intake profile indicative of higher risk for cardiac disease. These findings suggested that olfactory dysfunction may make it more difficult for elderly women to maintain a diet to control for risk of chronic disease. The authors recommended nutritional counseling for elderly patients with olfactory dysfunction. In a study by Mattes and Cowart, patients with chemosensory disorders and controls were evaluated for disorder-related changes in food habits, nutrient intake, and body weight[8]. A broad variety of dietary responses to chemosensory disorders was identified. Patients with multiple chemosensory disorders were most likely to lose weight, while anosmics demonstrated a tendency to gain weight. They also concluded that patients with chemosensory disorders may be at "nutritional risk." This risk includes potential overcompensation for loss of olfactory stimulation from foods by increased use of sugar and seasonings, seen in 20 - 40 % of patients with anosmia[8,9]. This may lead to difficulty, particularly in patients who must carefully monitor their intake of sugar or salt, such as diabetics or hypertensives.

> "I am considerably more anxious in day to day activities… anxious about being alone because I may not be able to prevent some home accidents." - 51 year old female homemaker with reduced and distorted olfaction from sinonasal disease

Financial Implications

There is no available data on the financial impact of olfactory loss. However, olfactory dysfunction may have a financial impact on both the individual experiencing the loss, and society as a whole. This could arise from a number of different factors.

First, individuals in a number of professions rely heavily on their sense of smell for their daily functions, and would thus be comprised in their occupations in the event of an olfactory loss. Cooks and chefs would be unable to sample their work to assure its palatability, or to monitor stored ingredients for spoilage. Firefighters might have considerable difficulty locating hidden fires if their ability to detect smoke were impaired. Daycare providers or babysitters with impaired olfaction would be unable to detect soiled diapers, leading to hygiene and health problems. Similarly, wine or food tasters and critics, chemists, and perfume and fragrance designers and retailers all rely heavily on their sense of smell for their livelihood, and might be unable to function following an olfactory loss. Other professions, including electricians, natural gas workers, mechanics, and even healthcare providers rely on their sense of smell to some degree in their work, and thus would likely experience reduced efficiency or performance in the

workplace with olfactory impairment. Thus workers in such "olfaction-dependent" professions may need special consideration when considering their level of impairment following an olfactory loss. Certainly the 1 % to 5 % level of impairment assigned to olfactory loss by the AMA *Guides to the Evaluation of Permanent Impairment* underestimates the true impact of olfactory loss on these individuals.

Second, patients with impaired olfaction might be less likely to purchase items or engage in activities that appeal to the sense of smell. Thus this group as a whole may spend less money on fragrances, certain food items, dining out, or fresh flowers, having a small but real impact on these industries.

Lastly, there is clearly a direct cost attributable to the evaluation and treatment of patients with olfactory loss. This might include the costs of evaluations by several medical specialists, a variety of laboratory, radiographic, and olfactory function tests, trials of a number of over the counter or prescription medications, as well as possibly sinonasal or other surgical procedures.

Quality of Life

Multiple studies have shown that patients with olfactory dysfunction report lower quality of life. Deems et al demonstrated that the prevalence of depression in patients with chemosensory dysfunction

> "I eat but don't enjoy. I think I eat a lot more in hope I can taste the next bite." - 75 year old female homemaker with severely impaired olfaction from unknown causes

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was higher than in controls[10]. In a previous study from our institution, 420 patients who had undergone testing for chemosensory complaints were surveyed regarding the impact of their olfactory loss on specific activities of daily living, as well as quality of life issues[4]. Subjects with olfactory dysfunction reported greater impairment compared with controls in a number of olfaction-related activities such as eating, detection of gas leaks or fires, and housecleaning, as well as a number of potentially non-olfaction-related activities such working, socializing, and sports or exercise. Overall satisfaction with life correlated with patient-reported level of olfactory function. Patients with olfactory impairment reported increased concern about gas leaks, fires, and exposure to harmful substances compared to controls. In addition, patients reported less enjoyment of eating, and more concerns about personal hygiene. In another recent study of

278 patients with olfactory deficits, an average 20 % decrease in quality of life was reported, although figures differed depending on the cause of the loss[5]. In addition, patients reported a variety of difficulties in activities of daily life - 73 % reported difficulties with cooking, 68 % mood changes, 56 % decreased appetite, 41 % impaired perception of their own body odor, and 8 % problems at work. Other studies have demonstrated similar deleterious effects of olfactory dysfunction on patients' sense of well being[11-12].

Patient Counseling

The broad spectrum of sequelae of olfactory dysfunction, and the paucity of effective treatments together underscore the importance of counseling for patients presenting with olfactory disturbances. Patients with olfactory dysfunction need to be informed about the specific risks associated with their deficit, with particular regard to the potential impact on their occupation. Patients should also be advised to institute protective measures to avoid potentially life-threatening events. Installation and regular maintenance of smoke and natural gas detectors is of paramount importance. Similarly, regular inspection and maintenance of natural gas appliances is recommended. Written dating of perishable food items, and their routine inspection by a non-olfactory impaired friend or family member should minimize the potential for food poisonings. These seemingly simple measures, although not curing the underlying disorder, help reassure patients, lessen their risk of life-altering consequences, and thus improve their perceived quality of life.

Summary

A widespread lack of awareness and understanding among both the medical and lay communities causes olfactory disorders and their impact to frequently be overlooked. However, patients with olfactory impairment may experience a significant degree of disability in their daily activities including their occupation, increased risk of accidents such as fire, inhalation exposures, or toxic ingestions, dietary alterations impacting health, and overall reduced quality of life. Although for most olfactory disorders treatment options remain limited, enhanced physician recognition and counseling of patients with olfactory complaints may help to improve these patients' lives and lessen the impact of olfactory impairment on both the individual and society ■

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Taste and Smell Disorders: A View from Clinical Practice

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Of all the senses, smell and taste function still remains the least understood. In the last ten years great strides have been made in understanding the anatomy and mechanism of action of smell and taste in health and disease.

In clinical practice, disorders of smell and taste mostly involve testing, diagnosis and education of patients. Definitive treatment has not been available for many of these disorders except sinus infections, nasal polyps, medication side effects, zinc deficiency etc. Many patients continue to have disturbances of quality of life, including loss of appetite, weight alterations, and depression. It's not that testing, diagnosis and education do not help alleviate worry and anxiety, and give reassurance to many, but other treatments or compensatory mechanisms would be very welcome.

I am a neurologist in private practice in Houston, Texas, and now in Austin, Texas, for the last thirty years. I have been working in my Taste and Smell Disorders Clinic for nine years. My interest in taste and smell initially began with neurological treatment of Alzheimer's dementia, and clinical recognition of taste and smell disorders in these patients. My interest mushroomed to other neurological disorders like head trauma, and non-neurological disorders such as viral infections and side effects of medication.

I have also noted in my practice that elderly patients with various neurological complaints often have associated weight loss, with and without loss of appetite, often unexplained or blamed on medication side effects. In some cases, cancer workups are done, and in others, some or all offending medications are eliminated. Disorders of smell and taste are frequently overlooked as a cause.

The one compensatory treatment mechanism of some taste and smell disorders that has been recognized, but requires more study, and in my experience is infrequently discussed with patients, is food modification and preparation. Patients with moderate to severe smell loss have the inability to recognize flavors, but still have normal taste sensation (sweet, sour, bitter, salt and umami), and trigeminal nerve function (temperature, texture and spices). Therefore, with modification of food preparation to take advantage of this, patients can improve their appreciation for food. Patients with mild smell and taste loss can also benefit from food modification, but especially benefit further by hyperconcentration of tastants and flavors.

In my experience, most patients do not follow through with these principles of food modification and preparation because they lack explicit recipes to follow and/or the difficulties of having to prepare different meals for the rest of the family. In my practice, I am beginning a project, with the help of a chef and dietary specialists, to develop a series of recipes that are easy to prepare, but specifically target patients with taste and smell disorders of different severity.

To give some idea of how taste and smell disorders interfere with a patient's quality of life, I reviewed cases from my Taste and Smell Disorders Clinic that I have seen over the last eight months. I have purposely omitted many neurological disorders like Parkinson's disease or Alzheimer's disease because these patients usually do not complain of smell or taste disorder and often deny that there is a problem. Primarily, I was looking at the patients who are symptomatic. On average, I had seen these patients within nine months of onset of their symptoms. A total of 44 cases were reviewed. They include 21 females with a median age of 56, and 23 males with a median age of 63. The causes of their smell and taste disorders were as follows: viral 13, head trauma 6, unknown 14, medication side effects 9, sinus problems and nasal polyps 3, and post cerebral rupture 1. Nine had a primary taste disorder and 35 had a primary smell disorder.

The test that I used for smell was the University of Pennsylvania Scratch and Sniff Test that is standardized for age and gender. Nine had normal smell to mild anosmia and 35 had moderate to severe anosmia.

Appetite is a very important aspect of taste and smell disorders, and information was available in thirty-five cases. Seventeen reported normal appetite, ten had mildly decreased appetite, and four had moderate to severe decrease in appetite. However, four had an increased appetite. It appears, from reviewing these cases, that over half of these patients had an appetite disturbance. The four that had an increased appetite stated that they ate more because they were not satisfied by the food that they were eating. They indicated they were always looking for a better taste to appear.

Thirty patients reported on the actual quality of their taste. Twenty-two stated that the quality was "very distressing" or "horrible", and eight

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Taste and Smell Disorders:
A View from Clinical Practice continued

reported "mildly distressing" to "normal" taste. In another 25 cases, 13 described the taste of food as "bad to horrible", and 12 described "normal to fair". Weight changes that were felt due to the smell and taste disorders: 26 no change, 3 weight gain, and 5 weight loss (average 10 pounds).

I looked at the presence of depression, as it appeared to be related to the taste and smell disorder. Out of 30 cases where the information was available, 19 had no depression and appeared normal, five had mild depression, six had moderate depression, and one had severe depression.

If you ask ordinary patients (not those taste and smell affected) where they place taste and smell sense in importance, on a scale of 1 to 10, as compared to other senses such as hearing and vision, they usually give taste and smell a four, while they give hearing and vision a nine or ten. When I asked the same question of patients who have problems with their smell and taste, at least half gave smell and taste a nine or ten.

In summary, it appears that 50% of the patients have an impaired quality of life due to the smell and taste disorder. The majority are able to maintain their weight with a normal appetite in spite of the fact they do not enjoy eating. Some will develop weight loss or weight gain. I have used changes in food preparation and food modification with many of my patients and my feedback has been very positive (in improving their quality of enjoyment in eating and appetite).

The majority did not have depression, but 20% required extra reassurance, counseling and mild anti-depressants.

Of interest, two patients who were found to have moderate to severe anosmia on the smell test did not complain of any taste disturbance. In my experience, this is extremely unusual, and the food preparation and modification did not appear to be necessary. Why this is the case in these patients is unclear.

All cases of moderate to severe anosmia were unable to smell smoke, gas fumes, body odor, spoiled foods, perfume or deodorant, which negatively impacted their life and caused some anxiety. A few of my younger patients were distressed that they could not smell their child's dirty diaper and when it needed changing.

Some patients with irreversible symptoms of smell and taste disorders can benefit from changes in food preparation, counseling and anti-depressant medication, all of which, in my experience are under utilized and under recognized in clinical practice. Further work on this subject is needed. Elderly people who develop unexplained weight loss and decreased appetite should be carefully questioned and studied for smell and taste problems. The old cliché that you don't appreciate something until it is gone applies very well to taste and smell disorders ■

ABSTRACTS:

OLFACTION TARGETED

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The olfactory system provides sensory information about the chemical composition of the external world. Olfactory chemoreception initiates in mammals at the level of sensory neurons that are located in the main olfactory epithelium and the epithelium of the vomeronasal organ (VNO). The former mediates mainly the detection of volatile odorants. The VNO mediates mainly the detection of non-volatile odorants, many of which are pheromones. These are chemical signals that provide information about gender, dominance and reproductive status between individuals of the same species.

The dichotomy between the main and vomeronasal (or accessory) olfactory systems is further reflected at the level of the molecules that serve as receptors, or putative receptors, for their respective sensory stimuli. In the main olfactory system, odorant receptor (OR) genes encode seven-transmembrane proteins and are members of a multigene family that may comprise as many as 1000 genes in mouse and human. In the accessory olfactory system, two families of genes encoding seven-transmembrane proteins have been proposed to encode pheromone receptors. The first family of vomeronasal receptor (VR) genes is expressed selectively in neurons of the apical zone of the epithelium of the VNO. The second family of VR genes is expressed in neurons of the basal layer. There are no conserved motifs between the two families of VRs, and VRs have no sequence homology with ORs.

These chemosensory receptors are encoded by some of the most complex gene repertoires in the mammalian genome. Mining the Celera and public databases, we composed a first near-complete draft of the mouse V1R repertoire, cataloguing 137 intact genes in 12 distinct families. Our exploration of the human V1R repertoire resulted in the discovery of the five human V1R genes with an intact open reading frame. Axons of neurons expressing a given V1R or V2R converge onto numerous glomeruli in the accessory olfactory bulb. Interestingly, dendrites of second-order neurons (mitral cells) frequently project to glomeruli of the same type. Thus, the initial divergent pattern of projections is rendered convergent in the accessory olfactory bulb.

DELETION OF OLFACTORY MARKER PROTEIN (OMP) CAUSES ABERRANT AXON TARGETING IN THE OLFACTORY BULB.

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Olfactory marker protein (OMP) is expressed by mature primary olfactory sensory neurons during development and in adult mice. In mice that lack OMP, olfactory receptor neurons have perturbed electrophysiological activity and the mice exhibit altered responses and behaviour to odor stimulation. To date, defects in axon guidance in mice that lack OMP have not been detected. During development of the olfactory system in mouse, primary olfactory axons can often overshoot their target layer, the glomerular layer, and project into the external plexiform layer. These normally occurring aberrant axonal projections are detected within the external plexiform layer up to postnatal day 12, after which primary olfactory axons are essentially absent from the deeper layer of the olfactory bulb. We have examined at the single cell level, the projections of primary olfactory axons in OMP-tau-lacZ mice, in which the OMP coding region has been replaced by tau-lacZ. We have found that axons over-project past their target layer into the deeper layer of the bulb, the external plexiform layer. The over-projecting axons are present up to 5 weeks postnatally, and thereafter the number of aberrantly projecting axons decreases. However, even at 8 months aberrant axons could be detected. In heterozygous mice, axons also over-project into the external plexiform layer, however there are fewer axons and they project for shorter distances compared to their homozygous littermates. Our results suggest that perturbed electrophysiological responses caused by a loss of OMP in primary olfactory neurons reduces the ability of primary olfactory axons to locate their glomerular target.

This work was supported by grants from the National Health and Medical Research Council to J.St.J and B.K., and by a grant from the Garnett Passe & Rodney Williams Memorial Foundation to J.St.J.

INTEGRATING THE MOLECULAR AND CELLULAR BASIS OF ODOR CODING IN THE DROSOPHILA ANTENNA

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The odor-specificities of olfactory neurons form the basis of the olfactory code of an organism and are believed to depend on the odorant receptor (Or) proteins expressed in these neurons. In *Drosophila*, isolation of the *Or* gene family and functional identification of discrete olfactory receptor neuron (ORN) classes by electrophysiological analysis allowed us to start establishing a correspondence between individual receptors, neurons and odors. We have demonstrated that individual receptors map to individual neuronal classes through a genetic and molecular analysis of two *Or* genes, *Or22a* and *Or22b*. We have shown that these receptors are localized in the dendritic membranes of ORNs in a subset of olfactory sensilla on antenna. By GFP-labelling or ablating the ORNs expressing Or22a and then performing electrophysiological recordings, we have mapped Or22a to a single functional class of ORN, ab3A, and by extension to the odors to which ab3A is sensitive. These results are confirmed by analysis of a mutant lacking *Or22a* and by rescue experiments with *Or22a* transgenes. The broad response spectrum of the ab3A neuron is accounted for by a single receptor: while we found evidence that *Or22b* is co-expressed with *Or22a*, *Or22b* is neither necessary nor sufficient for ab3A function. Ectopic expression of a different receptor in a mutant ab3A neuron has been used to identify the neuronal class in which this receptor is normally expressed, and to demonstrate that the identity of an ORN depends on the *Or* gene that it expresses.

CHARACTERISATION OF OLFACTORY PROGENITOR CELLS AND NEUROSPHERES USING TIME-LAPSE VIDEOMICROSCOPY AND IMMUNOCYTOCHEMISTRY

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The mammalian olfactory neuroepithelium is recognised as possessing a unique stem cell as it is capable of reconstituting cells of both neuronal and non-neuronal lineages after injury, and it avidly supports neurogenesis in the normal adult. Our understanding of olfactory neurogenesis remains limited, however, and the olfactory neuronal stem cell has not been unambiguously identified. We reasoned that isolation and full characterisation of this cell might reveal the basis for the region's striking permissiveness for neurogenesis, as well as providing a valuable source of proliferative cells for stem cell grafting. Putative stem/progenitor cells were enriched by selective filtration away from a preparation of dissociated primary neonatal rat olfactory neurones (Cunningham et al., 1999). Using time-lapse videomicroscopy we identified single, motile cells that generated "neurospheres". Immunocytochemical (ICC) analysis of spheres at different stages of development *in vitro* was performed with general neuronal and glial markers, markers for olfactory neuronal, sustentacular and basal cells, and nestin. Double-labeling ICC analysis of individual spheres revealed heterogeneity, consistent with cell specification occurring early in the development of the clonal neurospheres. Analysis of spheres stimulated to differentiate confirmed that their neuronal progeny were of the olfactory lineage. Cells in the spheres were passaged and generated secondary spheres, although at lesser frequency than CNS neurospheres. The growth characteristics, differentiation and progeny of olfactory spheres were directly compared to those of central neurospheres generated from neonatal forebrain. Our novel *in vitro* system of olfactory neurospheres provides a model of neuronal differentiation that will allow better understanding of the earliest stages of olfactory neurogenesis.

Supported by the Garnett Passe & Rodney Williams Memorial Foundation

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SENSORY EVALUATION, BY CHILDREN AND ADULTS, OF A COMMON FOOD PRODUCT IN THE U.K., TWO REGIONS OF ITALY AND MEXICO.

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Food companies contemplating expansion of sales into new export markets face the decision of whether to find markets where their current products will be acceptable, or to go to the expense and risk of tailoring products for each market. Sensory attributes driving acceptance have been shown to have common and distinctive differences, over a range of products in Japan, Indonesia and Australia (Bell & Song, 1998, *Chemical Senses*, 29(2) 240P). If the same product is evaluated across different cultures, the effect of culture on the perception of attributes and drivers of acceptance should be able to be determined with increased precision. The food manufacturer can then see which countries or regions might offer the best opportunities for the product.

This study took the same three variants of a manufactured food into three countries and applied identical methodology of assessment to groups of 40 children (8-12 yrs old, equal numbers of males and females) and 25 adults (females). The UK groups were recruited in Coventry, England. Two Italian regions were visited: the South, represented by the city of Latina, which lies between Rome and Naples, and the North, represented by Milan. The Mexican groups were recruited in Mexico City. The foods were assessed on unstructured graphic line scales bounded by a descriptor at each end. The products were presented blind (without packaging), in randomised order, one sample at a time. The children assessed 5 attributes and the adults 25. Although the products were liked to varying degrees across the four country/regions, there were four drivers of liking common to all in the adult groups. The UK children's' group had no statistically significant drivers, but in the other three children's' groups three out of the five attributes were correlated significantly with liking. UK and Southern Italian children and adults liked the product to the same degree, whereas Northern Italian and Mexican children liked the product significantly more than the adults did. The variants of the products were differentiated on several attributes but not on overall liking. The data provided the manufacturer with clear guidance as to which countries/regions to omit, as well as which markets, by region and age of consumer, offer opportunities for the existing, unaltered products.

Acknowledgment: Thanks to Christine Broughan, Gaye Wilkinson and Robyn Hudson for facilitating the fieldwork and recruitment. The study was performed under contract to The Centre for ChemoSensory Research.

VALIDATION OF YOGHURT PREFERENCES IN THE AUSTRALIAN MARKET

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This 2 stage study was designed as a comparison of consumer and trained panel responses to yoghurt leading to the development of new product.

The purpose of stage 1 of this study was to evaluate the sensory profile of a number of yoghurt products in order to optimise and provide direction for a client's current portfolio, as well as to identify any new product opportunities that may exist within the category. A methodology was employed whereby a variety of yoghurts were selected from the current Australian market, current overseas market and developed prototypes. A trained panel profiled each of the products, and then consumers evaluated the yoghurts for acceptability and other sensory properties. As consumers are good at identifying their overall acceptance (or liking) of a product but are poor at describing in detail why, the trained panel data and consumer acceptability data were combined together to highlight the product attributes most responsible for driving consumer acceptance. The trained panel data and consumer acceptability data were combined using regression and partial least squares analysis which was visually represented on a map. The analysis identified that the client's range was perceived to be too similar for consumers and did not maximise variety. In order to identify market gaps and opportunities, a conjoint analysis was utilised. The analysis identified 3 distinct cluster groups and the most motivating attributes driving each cluster's yoghurt preferences. Through understanding the conceptual market drivers, the client was able to develop prototypes which addressed identified gaps in their current range offering.

Stage 2 of this research involved the client developing a new product based on the research findings from stage 1. The client wished to confirm consumer acceptability of the new product by validating it against the original conjoint clusters defined in stage 1 of the research. A condensed version of the original conjoint was developed using key predictive scenarios which would enable the respondents to be quickly reclassified back into their original cluster groups. Would the same consumer preferences still exist?

CROSSING THE BOUNDARIES BETWEEN TRADITIONAL AND FUTURE SENSORY ANALYSIS FOR FMCG

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There is no need to tell you that the world is changing at a rapid pace. Consumers are changing and therefore our methods of understanding them must change. Most of the methods used today were developed for specific purposes that are no longer current in today's environment.

There are two 'buzz' words in the marketing world at present 'insight' and 'differentiation'. Traditional sensory research will in the future deliver insufficient insight to assist in brand differentiation. New methods that explore areas outside of the traditional taste test will be required.

Marketing and brand insights in the area of fast moving consumer goods are explored to demonstrate the type of information/insight that product development and marketing will be requiring of sensory research in the future.

TEMPORAL PROCESSING OF TASTE MIXTURES AFFECTS THE IDENTIFICATON AND ORDER OF PERCEPTION OF COMPONENTS

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The capacity of humans to identify the components of taste mixtures is limited to 3 (Laing et al 2002). Here the hypothesis that temporal processing differences ie differences in onset times, have a major role in limiting capacity is investigated. 32 subjects were trained to identify sucrose, sodium chloride and citric acid at 6 concentrations and indicate 1) which tastant was perceived first and 2) the identity of each component in binary and ternary mixtures. Concentrations of components were adjusted to provide a wide range of intensity conditions where in some instances time differences in processing the individual components were minimized. The results indicated that with *Binary Mixtures* systematic changes in intensity resulted in identification of only 1 component when the differences were largest, and both when intensities were similar. In contrast, subjects found it difficult to indicate which component was perceived first when the intensities were similar and easy when substantially different. More profound effects were found with *Ternary Mixtures*. With 8/18 mixtures, subjects could not indicate which component was perceived first, and in 15/18 mixtures not all components were identified above chance. The results support the hypothesis that temporal processing plays an important role in limiting the identification of tastants in mixtures. However, in contrast to odor mixtures where working memory and temporal processing were the two major factors limiting identification (Jinks, Laing and Hutchinson, 2001) suppression was proposed as the second major factor limiting identification of components in taste mixtures.

Jinks, A., Laing, D.G., Hutchinson, I. (2001) A psychophysical study of the relationship between temporal processing in odor mixtures and transduction pathways. Cognitive Brain Research. 12,273-288.

Laing, D.G., Link, C., Jinks, A., Hutchinson, I. (2002). The limited capacity of humans to identify the components of taste mixtures and taste-odor mixtures. Perception, 31, 617-635

The research was supported by an ARC Large Grant to DGL & a Centre For Advanced Food Research Scholarship to KM.

INFLUENCE OF DIETARY FATS ON TASTE PERCEPTION

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As well as being a significant source of energy, fats influence the flavour of foods by modifying its texture, odour and taste. Chemically, fats and oils are lipid molecules (triglycerides) which consist of three fatty acids attached to glycerol. Lipases hydrolyse triglycerides to release the fatty acids which are then metabolised to provide relatively large amounts of energy. The fact that fats contribute to one third of total energy intake in the Australian diet is a public health concern as both the total intake, and certain types of fats, are association with obesity, diabetes and cardiovascular diseases. In response to these concerns, and recognising the important sensory role of fats, several classes of fat substitutes have been formulated to mimic the texture provided by fats while minimising the energy contribution from fatty acids. However, while fat substitutes may provide similar textural characteristics as real fats do, it is much more difficult to mimic other flavour modifying effects of fats including possible taste effects due to interactions between fatty acids and taste receptors. Such a gustatory mechanism may be involved in our positive affective response to fats, and there is an accumulating body of electrophysiological and behavioural evidence to suggest that fatty acids do indeed stimulate receptors in the oral cavity. The objective of this psychophysical study, which used a trained panel of assessors, was to determine the extent to which various types of fats impact the one specific flavour modality of taste. Due to the simultaneous

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stimulation of multiple modalities (taste, texture, odour) when a food enters the mouth, texture-controlled model emulsions of deodorised fats were used as the base emulsion to which tastants were incorporated. The results showed that taste differences perceived by assessors depended on the type of oil used as well as whether the tastant was added at near-threshold or suprathreshold levels. ANOVA on the data showed that sunflower oil enhanced near-threshold sweet and salty taste intensities as well as suprathreshold savoury taste intensity at $p<0.05$. These results indicate that, apart from textural and volatile flavour effects, fats play a role within the taste modality by modifying intensity responses of specific tastants.

ESTIMATING THE "CONSUMER REJECTION THRESHOLD" FOR TCA IN WHITE WINE

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It is estimated that 5 - 7% of wines are tainted with 2,4,6-Tricholoroanisole (TCA). Also known as cork taint, TCA produces odors described as *musty, dank* or *earthy* that are unacceptable to many wines drinkers. Although previous estimates of TCA in wine have put the threshold as low as 0.5 parts per trillion (ppt), it is not clear at what levels TCA begins to render a wine unacceptable. It is therefore difficult, even if one knows the distribution of TCA levels in commercially available wines, to assess the economic impact of this wine taint. We developed a method to address this question by using a paired preference test within a typical method of limits threshold procedure. The aim was to determine the point at which wine consumers would begin to reject a wine containing TCA, which we termed the *consumer rejection threshold (*CRT). Fifty eight regular white wine consumers (Ss) received pairs of samples of white wine and were asked to taste each and indicate which of the samples was preferred. Ss received replicate series of 8 pairs, in which one wine sample was "spiked" with TCA at the following concentrations: 0, 0.5, 1, 2, 4, 8, 16, 32 ppt. To determine if the CRT was related to sensitivity to TCA, we also determined absolute thresholds (AT) for TCA in wine. Best estimate thresholds for the CRT and AT were 6.3 and 2.8 ppt, respectively, although this estimates are probably too high. Using criteria for significant differences for paired comparison tests gave values of ~3.7 (CRT) and ~2.4 (AT) ppt. CRT and AT were also significantly positively correlated (r = 0.43). Few of the Ss were "serious" wine drinkers as assessed by a wine knowledge test. Nevertheless, both CRT and AT were significantly negatively correlated with the score on a series of questions that assessed knowledge about TCA. A replication with a different group of consumers was carried out to ensure that carryover of TCA did not influence the outcome of the first experiment. This study gave a very similar result, with a CRT of ~3.9 ppt. These results provide a rational basis on which to assess the real impact of TCA in white wine, and estimate what levels of TCA should be regarded at unacceptable. The results also provide evidence for the utility of this method for determining CRTs.

PERSONALITY TYPES AND FLAVOUR PREFERENCES.

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Personal food choices and flavour preferences of individuals are influenced by many factors. Early studies focused mostly on demographic factors, such as culture/nationality as to why individuals have certain flavour preferences. However there is preliminary evidence to suggest that choices go beyond demographics, with links between personality types and flavour preferences/food choices. Colmar Brunton Research recognised the importance and value in this area, with outcomes allowing for more precise and personalised marketing. An initial literature review suggested that this is a relatively new area of research. The project therefore aimed to determine associations between Personality Types and Flavour Preferences, and investigate the implications on marketing. It was hypothesised that: Personality types influence the flavour preferences of individuals: The food a person chooses is reflective of their personality.

The research involved categorising a representative sample of individuals according to their personality types (using a psychological model and key ideas developed by Colmar Brunton), and collecting their preferences for a range of foods (chosen for their dominant taste characteristics). Associations between these two factors were then investigated and the impact this would have on marketing ascertained. The research outcomes reported assist in explaining why individuals make certain flavour/food choices and how this is associated with personality type and implied personal needs.

This research was supported by RMIT University, Melbourne VIC, Australia.

THE CAPACITY OF HUMANS TO IDENTIFY COMPONENTS IN ODOR-TASTE MIXTURES

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The capacity of humans to identify the components of taste mixtures or odour mixtures is limited to three (Laing & Francis, 1989; Laing et al., 2002), however, little is known about their capacity to analyse multi-component odour-taste mixtures. With one exception (Laing et al., 2002), previous studies have been limited to mixtures containing one odorant and one tastant. In the study by Laing et al, the odor-taste mixtures contained one odorant and one or two tastants. In each mixture with two tastants, the odorant was not identified, suggesting that taste may dominate smell when sampled in odor-taste mixtures as occurs during normal eating/drinking.

The present study aimed to determine whether the dominance of taste over smell in the earlier study was generally applicable to more complex odor-taste mixtures involving up to 6 components.

43 subjects were trained to identify 'equi' intense aqueous solutions of the tastants sucrose, sodium choride and citric acid, and the odorants (cinnamaldehyde, cinnamon), (cis 3- hexenol, grass-like) and (2-pentanone - like nail polish remover). Over 2 test sessions they were then asked to identify the components of 36 mixtures containing from 1 to 6 components. A solution was sampled using a straw from a 30 ml plastic cup which had a lid and a hole in the lid for the straw. Each subject sampled the solutions in a different randomised sequence. The results indicated that subjects were most easily able to identify components in 1 and 2 component stimuli but had decreasing success with mixtures containing 3, 4, 5 and 6 components. With each of these 3-6 component mixtures the mean maximum number of components identified was 3. Importantly, as reported by Laing et al., (2002), subjects identified tastes more readily than odors in the mixtures. It was concluded that increasing the number of modalities in a mixture does not increase the number of components identified. The limit of 3 components identified strongly suggests the limiting factor is working memory, the memory that is used to rapidly identify a stimulus and initiate a response within a second or two.

Laing, D G & Francis, G. (1989). The capacity of humans to identify odors in mixtures. Physiology & Behavior, 46, 809-814.

Laing, D.G., Link, C., Jinks, A., Hutchinson, I. (2002). The limited capacity of humans to identify the components of taste mixtures and taste-odor mixtures. Perception, 31, 617-635

This research was supported by an ARC Large Grant to DGL & a Centre For Advanced Food Research Scholarship to KM.

CROSS-STUDY ANALYSIS OF DIFFERENCES IN BRAIN ACTIVITY ASSOCIATED WITH LIKING RESPONSES TO DAIRY ODOURS

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Our sense of smell provides us with a great deal of information, most of which can be below our conscious awareness and therefore difficult to determine if relying on subjective responses. Objective techniques using electroencephalographic (EEG) have found that sensory responses relate to what our brain is doing at both a conscious and subconscious level. Some aspects of brain electrical activity changes must relate to processing of a flavour or odour, as evident with changes in EEG patterns when an odour is present or when the concentration of an odour is altered and even when the individual is not consciously aware of the presence of the odour.

The study reported was a cross-study analysis of differences in EEG associated with liking responses to 14 simple and complex dairy odours, with the aim to explore using such techniques to determine differences in flavour responses between different population groups, untrained in sensory analysis. Brain electrical activity responses to odours were compared with responses to subjective rating scales to determine differences in odour responses, independent of training and background. Based on Likert subjective rating scales, three *Liking* groups were formed from recordings for a total of 367 participant EEG odour sessions: *Like: n=131; Neutral: n=116; Dislike: n=120*. Using a One-Way ANOVA, brain activity frequency responses at frontal sites were examined for each liking response group. There were significant between-group differences in relative % power *difference* responses associated with Liking response in *beta* (13-16 Hz) activation associated with increasing liking for an odour. These beta responses were significant at all frontal electrode sites used for detailed analysis. This result demonstrated that the brain processes associated with the emotional response to the odours was robust in both the left and right hemispheres of the brain, despite the use of a range of stimuli (single components and complex mixes). As *beta* wave bands are commonly interpreted to be associated with changes in activation or arousal, the changes in right and left hemisphere *beta* responses may also be a reflection of shifts in attention associated with the emotional reaction to the odour. As a result of the significant findings of this cross-study analysis, using responses from 367 participants and 14 different odours, it can be concluded that changes in *beta* activation associated with odorous stimuli can be used as an indicator of emotional responses to the odour,

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independent of differences in cultural background and expertise.

This work was supported by the Dairy Research & Development Corporation

THE DEVELOPMENT OF A PSYCHOPHYSICAL METHOD FOR THE SENSORY ANALYSIS OF FOODS BY INFANTS

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With infants, the majority of sensory methods have been used to measure the hedonic aspects of infant responses to solid foods. This study involved the design and implementation of a modified habituation-dishabituation technique to identify infants' ability to discriminate solid foods.

Healthy infants (n ~ 64) at the stage of weaning (3-10months) were fed pureed baby foods (donated by H.J. Heinz Co.) over a 10 day period. The first stage (Day 1-5) involved familiarising an infant to a new food by increasing their acceptance of the food. This involved the infant being presented with the same food (familiar food) at the same time each day for five days, with acceptance determined by an increase in food intake. Following familiarisation a modified habituation-dishabituation procedure was conducted whereby infants were fed a single spoonful of a test food (a food that was new to the infant) (dishabituating stimulus) several times during the feeding of the familiar food (habituating stimulus). Discrimination was subsequently measured by changes in facial behaviours to the test food. The initial measure of discrimination involved feeding the infants with six spoonfuls of the familiar food prior to the first presentation of a test food (spoonful 7). The remaining test spoonfuls were randomised over the remainder of the feeding session which consisted of 50 spoonfuls in total. This procedure was carried out on three separate days (day 6, 8 and 10) using a different test food each day (The test foods had differing sensory characteristics compared to the familiar food in order to determine the basis of discrimination). The facial movements of infants in response to these foods were recorded using a video camera in the home. The infants' facial movements were analysed by an independent observer using a procedure incorporating facial features from Steiner (1979), Ekman and Friesen (1978) Facial Action Coding System (FACS) and Oster and Rosenstein (in press) Baby FACS. The independent observer used the Observer Video Tape Analysis System (Noldus 1995) to code the observed facial movements for a single spoonful presentation of the familiar and test foods. Two segments of the habituation-dishabituation feeding paradigm were assessed, with the observation data analysed using McNemar's Test of Symmetry (Siegel 1956). The initial segment assessed infant responses between the first test food presentation (Test Spoon 1) and the preceding familiar food presentation, with difference in facial behaviours between these two foods indicating discrimination. The majority of facial expressions displayed more in response to the test food than the familiar food represented negative hedonic behaviours, which were not specific to a particular sensory characteristic as they were common to all three test foods. Hence, infants responded to the novelty of the test food presentations.

This study has provided a basis for future research in assessing the sensory capacities of infants in relation to chemosensory stimuli and in turn determine the role of the senses in the development of food preferences. The commonly displayed behaviours could be used to determine when an infant perceives a change in a sensory feature, eg. orange to lemon, sweet to bitter, etc.

DEVELOPMENT OF CLINICAL TESTS OF SMELL FOR CHILDREN

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During the past decade or so, a significant effort has gone into the development of clinical tests of smell for adults. The loss of smell at the early stages of both Alzheimer and Parkinson's disease, and the potential of a smell test as a diagnostic tool for these diseases, has accelerated the number of clinical studies on the sense of smell. Unfortunately, clinical tests for children, particularly young children have not been developed and there are few reports of clinical studies with children. In view of the absence of appropriate clinical tests of smell for children a research programme has been initiated in this laboratory, which aims to develop tests of smell for school-age children that are reliable, will identify children with disabilities in these senses, and the extent of the disability. Such knowledge should lead to appropriate treatments and management of smell disabilities, and the counselling of parents and other carers as regards how best to assist a child in relation to health, diet/nutrition, social and everyday activities.

The study described here compared the abilities of adults (n = 232) and children (n = 240) aged 16-75 years and 5-11 years, respectively, to identify 11 common odours that had been demonstrated previously to be identified by adults at levels exceeding a criterion of 90% correct responses for each odorant (unpublished work, Laing). Odorants were presented in opaque non-odorous squeeze bottles. The test procedure required subjects to sniff the outlet of the bottle whilst it was being squeezed by a tester (for 5-6 year olds) or themselves, and indicate from a list of 4 words which one best described the odor in the bottle. The results indicated, that as before, adults in the age groups 16-50 and 51-75 years obtained mean identification scores for each odorant that were > 90%. In contrast, all the yearly age groups of children failed to identify all 11 odorants at the criterion level and there was a steady decrease in correct identifications as the age of the children decreased. 5 year olds, for example, identified only one of the odors, baby powder, above the criterion level, whilst 11 year olds identified 8/11 odors above the criterion. Interestingly, the results showed that the worst identified odor by children was cloves with scores of 59, 54, 58, 23, 31, 12 and 38% being recorded by 11 to 5 year old groups, respectively. This poor performance appears to be a reflection of the infrequent use of cloves or clove flavour in foods compared to a generation or two ago, and the infrequent need for dental fillings. The latter was the main basis of identification of cloves for 16-35 year olds in the earlier study by Laing. To overcome the poor identification of common odors by children, words and photographs of odorous objects are now being tested with a new set of odorants including those which produced ~70% correct identifications in the present study. In addition, a survey of odorants that are highly familiar to children aged 5-6 years is in progress.

RAPID ASSESSMENT OF HUMAN TASTE ANATOMY AND FUNCTION USING A DIGITAL CAMERA

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Quantitative assessment of taste function in humans can be achieved using both psychophysical and anatomical methods (Bartoshuk et al., 1994). The anatomical methods usually involve the use of a videomicroscope to assess the number and density of fungiform papillae on the anterior tongue. Good correlations have been obtained between taste function, particularly sensitivity, and papillae density. Furthermore, the classification of subjects as supertasters, tasters and non-tasters of Propthiouracil has been achieved through the measurement of papillae density (Hosako-Naito et al., 1996). Recently, we reported (Hutchinson et al., 2002) that children aged 8-9 years have a higher papillae density than adults, and that the papillae of the children are rounder and more consistent in shape. Taken with the data by Temple et al (2002) that the human tongue continues to grow until approximately 14 years of age, the different papillae density in 8-9 year olds compared to adults, indicates that the sense of taste has not reached a stable structure by mid-childhood. In an effort to determine when the human tongue becomes adult, we aim to compare the papillae density and number in children aged 5 to 18 years. Although this task can be achieved using videomicroscopy, the rate at which children can be assessed is limited to 1-2 per day because the children have to travel to the University laboratory and the videomicroscopy procedure is slow, requiring 30-60 minutes per child. Accordingly, to overcome the low assessment rate we have investigated the use of a digital camera (Nikon Coolpix 4500) in conjunction with computer processing (Adobe Photoshop 7.0), to replace the videomicroscope.

The potential advantage proposed for the camera was that if sufficiently sensitive, it should be possible to film the tongue of a subject in 5-10 minutes, and importantly its portability would allow the assessment of many children at a school during a day visit. Accordingly, many months of work could be achieved in a few days. To determine the potential of the camera, a study was conducted which involved a comparison of papillae counts using both the videomicroscope and the camera to assess the same subjects. 16 subjects participated, comprising 7 adults (25-38 years) and 9 children (8-9 years). Food dye was used to stain an equivalent region (6 mm diameter circle) of the tongue of each subject, and the region assessed using videomicroscopy (Segovia et al., 2002) and the camera. The latter procedure involved taking several photographs eg 4-5 per tongue, during 5-10 minutes with each subject. The recorded photograph was then processed using computerised grids in Adobe Photoshop which provided a magnification of 9x-16x (4.5 megapixels) equivalent to the videomicroscope. Statistical analysis of the data using t tests (paired two sample test for means) indicated that the papillae density of both adults and children was very similar for the videomicroscope (n = 12.0 pap/6mm) and the camera (n = 12.9 pap/6mm). The results indicate that the digital camera can replace the videomicroscope in measurements of papillae number and density providing a new and rapid method for the assessment of taste anatomy and function in humans. Importantly, not only can the camera be used in isolated environments such as schools, but it should also be usable at the bedside or outpatient clinics in hospitals for the assessment of the loss and recovery of taste function in patients receiving radiotherapy, chemotherapy and other treatments that affect taste function.

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The research was supported by a CAFR Scholarship to MS and funds for the camera from CAFR.

EXHALED BREATH ANALYSIS AS A METHOD OF DISEASE DETECTION AND MONITORING.

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Exhaled breath analysis is a new non-invasive method of detecting lung diseases and monitoring their progress and treatment. Exhaled nitric oxide (NO) is a mediator and marker of inflammation and it was hypothesised that it would be possible to detect respiratory diseases by analysing the exhaled breath. Asthma is known to cause inflammation of the airways, and it was hypothesised that gaseous markers of inflammation such as nitric oxide (NO) would be increased in the breath of asthmatic subjects. NO has been identified as being a mediator and marker of inflammation, thus it may be expected that this gas will be elevated in the breath of asthmatic subjects. Exhaled NO (eNO) was measured by a chemiluminescence technique, which utilises the reaction between ozone and NO to cause the release of a photon. Normal and asthmatic subjects were recruited from hospital and community populations.

We and others have now performed a number of studies which demonstrate convincingly that exhaled nitric oxide is elevated in the exhaled breath of untreated asthmatic subjects. These elevated levels return to normal when the asthma is treated by the use of glucocorticosteroids, and exhaled NO has been demonstrated to be a predictor of asthma control. Levels are increased by ingestion of the NO precursor, L-arginine, and decreased by inhalation of an NO synthase inhibitor. Viral infections and other lung diseases appear to cause an elevated level of eNO. In addition, smoking cigarettes either actively or passively, the ingestion of alcohol, and caffeine have all been shown to reduce the level of exhaled nitric oxide, indicating that these findings are not specific to asthma.

Other markers of inflammation can also be measured either in the breath or in breath condensate, and it may be possible to combine these variables to develop a disease profile. Breath analysis may be a useful tool for the detection of respiratory diseases and may assist the control of asthma by demonstrating the degree of inflammation that is present in the airways.

RIESLING WINE AROMA: RELATING VOLATILE COMPOSITION TO SENSORY DATA USING PLS REGRESSION MODELS.

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Twenty commercial Australian Riesling wines of varied ages, and sourced from a number of viticultural regions, were profiled by sensory descriptive analysis using a trained panel of 16 assessors in triplicate assessments. The same wines were also subjected to quantitative compositional analysis using a stable isotope dilution analysis GC-MS method applied to 45 volatile compounds, including grape-derived monoterpenes and norisoprenoids, and fermentation derived esters, acids and alcohols. The two data sets were related using partial least squares regression models. Of the sixteen aroma attributes that were rated as significantly different across the wines, satisfactory partial least squares regression prediction models could be developed for twelve, using a total of 28 volatile compounds. The attributes that could be predicted best from the chemical data included perfumed floral, pineapple, estery/bubblegum, honey, toasty, caramel and kerosene. The compound TDN, which is known as a contributor to a kerosene-like aroma in some older Riesling wines, was of particular importance to the prediction models, as were five esters, two monoterpenes (linalool and geraniol), and -damascenone. The results suggest that the determination of a relatively small number β of volatile compounds for a Riesling wine sample can allow an indication of the aroma properties of that wine.

CHEMICAL AND SENSORY ANALYSIS OF CHEESE AROMA COMPOUNDS USING SOLID PHASE MICRO EXTRACTION (SPME) AND GAS CHROMATOGRAPHY OLFACTOMETRY / MASS SPECTROMETRY (GS/MS GC-O)

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Gas Chromatography Mass Spectroscopy (GC-MS) techniques are commonly used for quality control and product development, providing industry with a profile of the chemical components in products. However, a key aspect of the flavour response is the aroma, and GC-MS does not necessarily have the sensitivity to provide information about the important impact compounds in an aroma. This paper reports analysis conducted on cheese aromas using Solid Phase Microextraction (SPME) for the concentration of cheese aromas for analysis by GC-MS and GC-olfactometry (GC-O) for the identification of the major impact aromas in cheese.

Nine commercially available cheeses were examined using solid phase micro-extraction (SPME). Three of each of the following cheese varieties were examined: cheddar, hard grating-cheese and mould-ripened blue cheese, all obtained from the local supermarket. Volatile components were concentrated using polydimethylsiloxane (PDMS)-Carboxen fibres (75 m) for 16 hour (overnight) prior to GC-MS and GC-O μanalysis. Odour components perceived at the olfactory port (OP) were matched with electron impact (EI) and methanol chemical ionisation (CI) mass spectra, and the data were compared to previously reported cheese aroma compounds.

Although hundreds of volatile compounds have been identified in the headspace of cheeses, only a small number contribute to actual cheese aroma. Of the aroma components identified *via* olfactometry, *methanethiol, methional, dimethyl trisulphide* and *butanoic acid* were present in all of the cheeses, implying their essential role in the formation of basic cheese aroma. A number of *alkyl-pyrazines* were found to impart roasted nutty, raw potato and savoury broth-like notes in some of the cheeses. In all cases, the aroma active compounds identified *via* this method were in broad agreement with those reported in the literature.

PDMS-Carboxen fibres were particularly effective in the concentration of important sulphur flavour compounds which have been cited as important components of cheeses aroma. Although both *dimethyl sulphide* and *dimethyl disulphide* were clearly present on the total ion chromatograms of all cheeses, they contributed only marginally to the cheese aroma. In contrast, the aroma intensity at the OP of both *methional* and *dimethyl trisulphide* were consistently high. Interestingly, dimethyl trisulphide with its sweet, savoury, garlic aroma is rarely discussed as a major component of cheddar flavour, although it has been previously detected in cheddar headspace.

Olfactory profiling demonstrated that some high impact odour compounds were close to or below the limits of instrumental detection, with significant key odour components not always easily evident on total ion chromatograms. The largest peaks identified by GC-MS often made marginal or no contribution to the cheese aroma, hence underlining the importance of GC-O as a complimentary tool for aroma analysis. This study serves to demonstrate the importance of incorporating GC-O aroma analysis techniques into quality control and product development, providing a greater understanding of the aromas contributing to the flavour experience.

Supported by the Dairy & Wine Research & Development Corporation Australia.

SENSORY PROPERTIES OF CHARDONNAY WINES PRODUCED FROM POWDERY MILDEW AFFECTED GRAPES

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The sensory properties of wine made from Chardonnay grapes with increasing levels of powdery mildew infection was assessed over two vintages (2001 and 2002). Wines were made using standardised experimental winemaking methods in replicate. For each of the two vintages sensory data was collected in separate studies. Discrimination testing and sensory descriptive analysis was carried out using in-house panels. Sensory data was also collected from a group of experienced wine industry personnel using less formal procedures: eight wines were presented as a single set at a single session, with no prior training of the tasters. The descriptive analysis results for the samples from the 2001 vintage showed that wine made from grapes with powdery mildew infection was rated significantly higher in 'oily' and 'viscosity' flavour attributes than wines

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made from disease-free grapes, with other attribute differences relating to differences in fermentation parameters or secondary compositional effects of the powdery mildew infection. For the 2002 vintage basic juice composition was more closely similar across treatments than was achieved in 2001, although there were some acid differences among the treatments. The wines from the powdery mildew affected grapes from this vintage were again rated by a trained sensory panel as higher in 'oily/viscosity', with the wine made from the grapes with 31-100% powdery mildew affected fruit from this vintage also rated significantly higher in 'fungal' and 'earthy' aroma attributes. The 'oily' attribute was found to be related to the concentration of phenolic compounds present in the wine in both years, with these compounds also correlated to 'fungal' aroma ratings for the 2002 wines ($r>0.9$, $P<0.002$, $n=8$). As expected, data from the winemaker panel was of less value in discriminating the samples, which was largely reflective of the task the tasters were asked to carry out.

WHAT THE NOSE KNOWS: PREFERENCE FOR HUMAN BODY ODORS AS A FUNCTION OF GENDER AND GENDER PREFERENCE.

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In preliminary work conducted by Crabtree, Preti and Wysocki (unpublished), it was noted that lesbians responded differently to human body odors obtained from heterosexuals and homosexuals - lesbians preferred the odor of underarm sweat from other lesbians relative to that from heterosexual females. Based on these results, we hypothesize that there is (a) a detectable difference in body odor based in part upon gender preference and (b) an influence of gender preference on the perception of and preference for the odors of underarm sweat from heterosexuals, lesbians, and gay males. In the present study, heterosexuals, lesbians, and gay men indicated their preference for one member of each of the following pairs of underarm sweat odors: (1) heterosexual male vs. gay male, (2) heterosexual male vs. heterosexual female, (3) heterosexual female vs. lesbian, and (4) lesbian vs. gay male. Odor pairs (collected from six members of each group and recombined to form odor classes rather than individuals) were presented 11 times each, counterbalanced across participants, in unlabeled, fragrance-free bottles. Results suggested a difference in the production of odor profile based upon gender preference and, at least for gay males, a preference for the odor from other gay males. Speculation on the underlying mechanism for differences in odor profile awaits the results of analytical assessments of collected sweat.

TOWARDS STRUCTURE-ODOUR-ACTIVITY-RELATIONSHIPS: ENHANCING THE ODOUR DEFINITION OF FRAGRANCE COMPOUNDS

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The structure-odour-activity-relationship [SOAR] term was invented by Helmut Hügel[1] who perceived that chemists could make a significant contribution to the understanding of the molecular mechanism of olfaction and to the industry by creating new synthetic fragrances. SOAR defines the odour active regions of a fragrant molecule and is a method of analyzing the molecular properties of fragrances. There are three variables: structure [3D shape], odour [type] and activity [molecular property] in the relationship. By systematically changing a chemical region of the molecular structure, the resultant odour change can be monitored and this can be related to interaction with olfactory receptor sites[2]. As a major study SOAR will be applied to the marine fragrance, a benzodioxepanone, Calone 1951. Changing the chemical constituents in regions A, B and C will alter the molecular interaction with the olfactory receptor and indicate how this recognition impacts on the odour. It has been hypothesized by Wang and coworkers[5] that metal ions [Cu^{+1}, Cu^{+2}, Zn^{+2}] are involved in odorant recognition and that the olfactory receptor [OR] is a metalloprotein. A metal-OR combination would be expected to show selective binding to odorant molecules based on the affinity to the metal and this has been found. We will use the SOAR analysis to investigate this hypothesis.

For the synthesis of Calone 1951 a prototype microwave reactor[6] was used. The parameters for successful microwave applications involves the presence of a permanent intramolecular dipole moment of the solvent or solid compound being irradiated. Dielectric heating required to stimulate chemical reactivity occurs as a result of coupling between molecular rotation and the oscillating electromagnetic field. Conventional heating methods rely on conduction and convection for heating of the reaction. This means the temperature gradient

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decreases from the edge of the sample towards the centre. In the case of heating using microwaves, the temperature gradient increases from the edge of the sample towards the centre[7], which obviously results in more effective heating. Conventional heating of the catechol to provide the diether(II) failed.

As part of the overall SOAR analysis, comparisons made using Gas-Chromatography-Olfactometry (GCO) will enable models to be established which define the olfactory active regions in relation to chirally active fragrances. The fragrant qualities of enantiomers will be determined using GCO, both in relation to each other and to the overall fragrance mode of the chiral compound. For some compounds only one enantiomer is active, indicating that the receptor site is also active[8]. When comparing compounds III and IV, it is apparent that the removal of the functional group of compound (III) increases odour strength and hence it can be assumed that the ester group may have a hindering effect when the molecule binds to the olfactophore receptor site. Other examples described[9] are compounds similar to Calone, but with an ether oxygen replaced with a carbon. The precursor to Calone 1951, exhibits chirality via an R and S form. As extended research, it would be feasible to isolate the two enantiomers and make comparisons between the odour characteristics of each.

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B.D. gratefully acknowledges the research scholarship received from the Faculty of Applied Science, RMIT University.

EVALUATION OF SOLID PHASE MICRO-EXTRACTION AND SIMULTANEOUS GC/MS-OLFACTOMETRY FOR THE CHARACTERISATION OF ESSENTIAL OIL AROMA COMPONENTS.

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Essential oils (EO) are made up of a complex mixture of mainly plant terpene hydrocarbons, most of which have marginal aroma value. The trace impact components responsible for the aroma often can only be reliably identified using olfactometry techniques in combination with GC/MS analysis. Traditionally there has been some difficulty associated with performance of olfactometry simultaneously with a mass spectrometer; generally olfactometry is performed with a flame ionization detector, separate to the GC/MS analysis. In this study we evaluated the relatively new ODO-II olfactory port connected to a GC/MS for simultaneous chemical and olfactory analysis. Solid phase micro-extraction (SPME) Carboxen adsorption fibres were used to concentrate the headspace of EOs.

A number of essential oils were examined including lime (*Citrus aurantifolia*), orange (*Citrus aurantium*), Indian Sandalwood (*Santalum album*) of dubious quality and Australian Sandalwood (*Santalum spicatum*). The oils were analysed by the traditional method (1) injection of the neat oil (1:100 split) and (2) by SPME headspace extraction with simultaneous olfactory analysis. The odour profiles from both techniques were compared. The adsorption behavior for various components was examined using varying SPME sampling times e.g. 1, 5, 15, 30 and 60 minutes. Varying sampling times indicated that SPME might be useful for developing a quasi-aroma extract dilution analysis approach to identify the major impact compounds in EOs.

ANALYSIS OF WINE AROMA USING SOLID PHASE MICRO-EXTRACTION AND SIMULTANEOUS GC/MS-OLFACTOMETRY.

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Carboxen Solid Phase Micro Extraction (SPME) fibres were assessed for their ability to provide concentrated wine aroma for simultaneous GC-MS-olfactometry experiments. Three white and three red wine varieties were examined; Chardonnay, Sauvignon Blanc, Semillon, Shiraz, Merlot and Cabernet Sauvignon. The concentrated aroma compounds were separated on a polar capillary column and the samples were split to the olfactory port and simultaneously to an ion-trap mass spectrometer. In all of the wines, red and white, the most intense aromas included *ethyl butanoate, ethyl hexanoate, methyl-butanol, beta-damascenone* and *2-phenylethyl alcohol*. A number of sulphur compounds, *dimethyl disulphide, methional, dimethyl trisulphide* and *methionol* also made contribution to the aroma profiles. Semi-quantitative analysis revealed some general differences between the red and white wines. Adsorption kinetics for various compounds was also considered in a preliminary study.

This work was supported by the Grape & Wine Research & Development Corporation ■

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WineSense:
To Cork or Not to Cork?

By Graham Bell

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Why are we seeing strange plastic blobs and screw-top closures appearing in the mouths of bottles of reputable wines? Are these not manifestations of poor quality product and low-cost packaging? What is happening to good old cork, the stuff of infinite variations in cork screw technology, the tug and the pop, the scrutiny and sniff of the wet end, the moment of decision, followed by the gurgle of wine into the glass?

There is a quiet revolution taking place across Australia, and in the wider world of wine consumption. Winemakers of high repute are turning to the screw-top as their closure of choice. Chris Hatcher, show judge and head of winemaking at Beringer Blass is one of those prepared to put his best products "under the screw": namely, the company's top red wine, Wolf Blass Platinum (retailing at around AUD$150) which has appeared in screw-tops since the 2001 vintage[1]. His company's other superior products, such as Annie's Lane Copper Trail Shiraz and Wolf Blass Gold Label Shiraz are similarly screw-topped from the 2000 vintages. These are among the best wines Australia can offer. They are at the opposite end of the spectrum to what we once used to pour from a screw-topped bottle (or flagon). Wine makers from large and small companies across Australia are turning the screw on cork closures. Chris Hatcher says "the problem with cork is not just cork taint (2,4,6-trichloroanisole, or TCA) and random oxidisation, but that it adds its own woody taste to the wine."[1]

This suggests that the market is heading towards rejecting high quality wines that are NOT screw-topped. What a strange turn of events we are witnessing, when in recent memory, only "plonk" ever appeared in a screw-topped bottle.

Meanwhile the cork suppliers must be in agony to see their centuries-old monopoly rapidly disintegrating. The company that controls most of the world's wine cork supply, Amorim, of Portugal, has, for the past three years made a multi-million dollar investment in improving the quality of wine corks.

They have launched, in recent years, at least two new types of wine cork (Twin Top® and Neutrocork®) and more recently, a special cleaning process based on controlled steam distillation, called ROSA. Enlisting the research support of British food research establishment, CCFRA, Germany's Geisenheim Research Institute, and Australia's AWRI, Amorim claims that "ROSA reduces releasable TCA in cork by up to 80%."[2] If so, can we expect, one in ten, a hundred or a thousand bottles to be of lower quality than the winemaker's product, before it went into the bottle? The data is not yet in.

And what do consumers think of the screw-caps? Do the new closures improve the quality of the wine on offer? Where is the consumer sensory research data on the question? No-one is saying, and the marketing gurus are strangely silent.

One thing is certain, more screw-topped bottles are appearing in my local bottle shop. They span a wide range of wine types, makers and prices. I am also encountering fewer "plastic corks", than year ago, and assume these are on the wane. These are impossible to see until it is too late and you have bent (detuned) another cork screw.

Among wine-loving friends I have interrogated, there is cautious acceptance of the screw-top. Usually they notice the top only at the time of opening it. So one form of packaging is not "speaking louder" than the other at the point of purchase. The quality of the wine that has been screw-closed, seems invariably good. Are we now experiencing, for the first time, wine as the wine-maker actually intended it to be, and not as the foibles of the cork determined it to be? If so, consumers need to hear this from the winemakers who are driving the decision to package under screw-caps.

In the long run, it will need proven, superior, perceived (sensory) quality of the screw-enclosed wine to win out against the nostalgia and fine old tradition of pulling, examining and sniffing a hallmarked cork. If Amorim's research can reduce, sufficiently, the taint and oxidation problems in cork closures, it will become increasingly difficult to make the sensory discrimination, and choice of closure will then be decided on issues such as cost, convenience, and appearance.

If it boils down to appearance and history, it will be difficult not to prefer the beauty and tradition of cork. On the other hand, if cork cannot win, or neutralise, the sensory flavour issue, then its days are numbered ■

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Upcoming Events

18 - 19 February, 2004	**Food and Allergen Forum and Workshop** Sydney Contact: Julie Bennett, + 61 2 8399 3996 aifst@aifst.asn.au
28 March - 1 April 2004	**Enviro 04 Convention & Exhibition** Theme: Advances in Odour Management: Towards More Livable Communities Sydney, Australia. (See details page 15) Integrated with meetings of CASANZ, AWA, WMAA, BCSE & EBA Info: www.enviroaust.net or margaretmetz@pacific.net.au
21-25 April 2004	**AChemS,** Sarasota Florida USA http://www.achems.org
6-26 June, 2004	**Chemosensory Neurobiology in the Marine Environment** Summer Course at Bermuda Biological Station for Research Info/applications: cderby@gsu.edu
5 - 9 July, 2004	**XIII International Symposium on Olfaction and Taste (ISOT) / JASTS** Kyoto, Japan Jasts@hus.osaka-u.ac.jp
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/

ChemoSense (ISSN 1442-9098)
Web: http://www.chemosensory.com
Published by **Graham Bell and Associates Pty Ltd**
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Ph. (+61 2) 9209 4083 ; Fax (+61 2) 9209 4081

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Editor: Graham Bell, g.bell@atp.com.au
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Design and Layout: Lawton Design Pty Ltd

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