Vol 14, No.1 December 2011

# Chemo sense

EDITORIAL

## Diagnose with Artificial Olfaction

By Graham Bell
*g.bell@e-nose.info*

**Chemical sensing** technology is moving ahead in the field of clinical medicine, both human and animal. In this issue, Boys, Thomas and Yates carry the technology forward in a field where it is badly needed: respiratory disease. Respiratory ailments burden a large percentage of the population and strike down both young and old. Experiments reviewed here show that progress is being made in rapid diagnosis using an electronic nose. With improvements in hardware and decision algorithms, the technology is set to achieve reliable early diagnosis, leading to better treatment and timely, life-saving interventions.

Miss the 2011 AACSS scientific meeting in New Zealand? Read the abstracts from the meeting, in this issue ■

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# On the Scent of GORD

## Electronic Nose Profiling and Exhaled Breath Condensate Collection in the Diagnosis of Gastro-Oesophageal Reflux Disease

*Emma Boys[1], Paul Thomas[2] and Deborah H Yates[3]*
*emma.boys@unsw.edu.au*

[1]Faculty of Medicine, University of NSW, Sydney, Australia
[2]Respiratory Medicine Department, Prince of Wales Hospital, Sydney, Australia
[3]Thoracic Medicine Department, St Vincent's Hospital, Sydney, Australia

**Gastro-oesophageal reflux disease (GORD)** is the most common upper gastrointestinal disorder in the Western world and has been associated with respiratory conditions including chronic obstructive pulmonary disease (COPD) and asthma. Current diagnostic standards for GORD are lacking in sensitivity and specificity, or are invasive and time-consuming. Novel methods such as the use of exhaled breath condensate (EBC) and electronic nose profiling have the capacity to improve current diagnostics. Improved diagnosis and treatment of GORD could improve quality of life for those with this common disorder.

The Montreal Consensus (2006) defines GORD as the presence of troublesome symptoms

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# On the Scent of GORD Electronic Nose Profiling and Exhaled Breath Condensate Collection in the Diagnosis of Gastro-Oesophageal Reflux Disease

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and/ or complications in the presence of retrograde flow of gastric contents into the oesophagus. The use of the word "troublesome" is defined as the presence of mild symptoms for 2 or more days per week, or moderate to severe symptoms occurring on one or more days per week for use in population based studies. Clinically however, what constitutes "troublesome" should be determined by the patient (Vakil et al, 2006). GORD is the most common upper gastrointestinal disorder in the Western world with weekly symptoms reported in 14-20% of American adults (Locke et al, 1997; Kahrilas, 1996). Risk factors include family history, BMI > 30, consumption of > 7 units of alcohol per week and tobacco use (Locke et al, 1999). There is also an association with irritable bowel syndrome, peptic ulcer disease (Ruigomez et al, 2004), dysphagia, dyspepsia, anxiety and depression (Locke et al, 1999).

### Laryngeal, Pulmonary and Oesophageal Manifestations

Although rarely a cause of mortality, approximately 30% of GORD sufferers have erosive oesophagitis, which may progress to strictures, intestinal metaplasia and Barrett's oesophagus, a major risk factor for oesophageal adenocarcinoma (Vakil et al, 2006). Evidence also links reflux with laryngopharyngeal neoplasia (Copper et al, 2000, El-Serag et al, 2001, Vaezi et al, 2006). Numerous studies to date have failed to clarify the exact relationship between respiratory diseases and GORD. However, associations exist with conditions including asthma, COPD and idiopathic pulmonary fibrosis (Fennerty, 1999) (Figure 1).

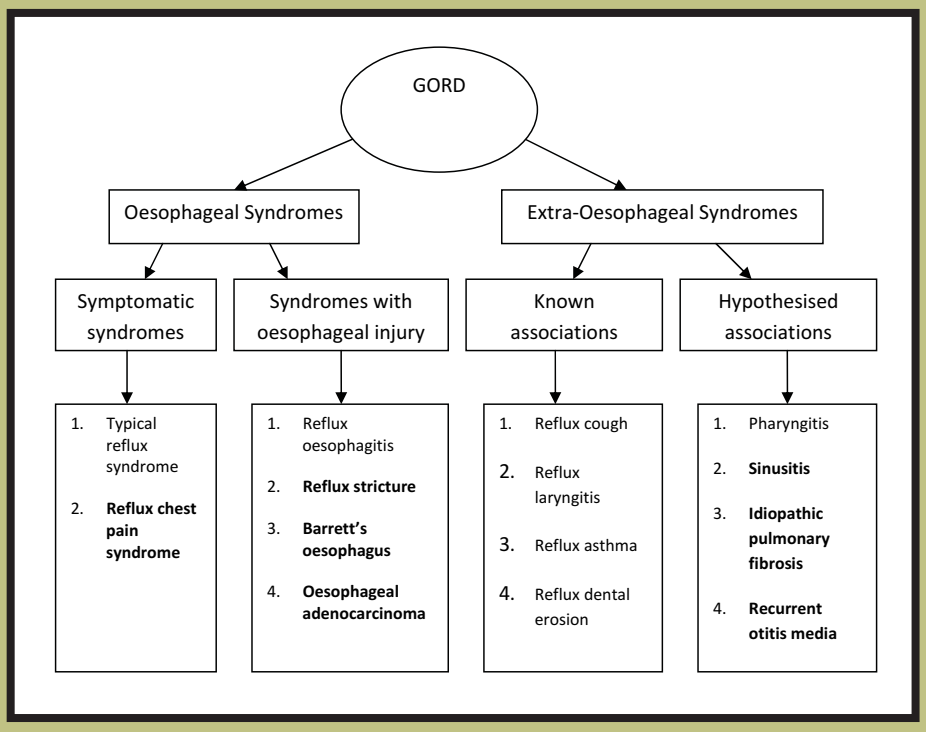


Figure 1: GORD and its current known and hypothesised associations. Adapted from Vakil et al, 2006.

*Two mechanisms have been proposed for the contribution of reflux to pulmonary manifestations (Figure 2). The first concerns microaspiration of stomach contents into the airways. The second is postulated to involve bronchoconstriction due to a vagal reflex arc from the oesophagus to the lung (Sontag, 2000), secondary to the common neural innervation shared by the oesophagus and lungs due to their common embryonic origin (Fennerty, 1999).*

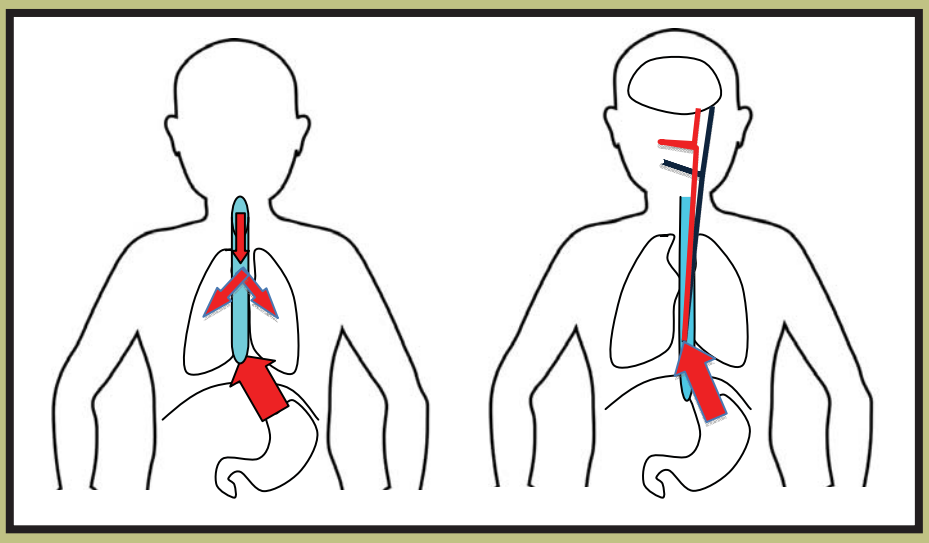

Figure 2: *The pathophysiology of extra-oesophageal manifestations of GORD. The left hand side demonstrates the direct action of gastric refluxate leading to inflammation in the airways. The right hand side demonstrates the vagally mediated-reflex whereby reflux into the oesophagus stimulates a neural arc leading to bronchoconstriction.*

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# On the Scent of GORD Electronic Nose Profiling and Exhaled Breath Condensate Collection in the Diagnosis of Gastro-Oesophageal Reflux Disease

continued

Repeated studies have demonstrated a statistically significant increased prevalence of GORD symptoms in asthmatic (65-80%) compared to control subjects (Field et al, 1996; Perrin Fayoelle et al, 1980; Sontag et al, 1990). Surprisingly, a recent Cochrane review reported that anti-reflux treatment did not lead to improved lung function or reduction in asthma treatment or symptoms. Possible explanations for these findings relate to the definition of GORD in that these subjects were not recruited specifically for reflux-associated asthma symptoms, and also that symptomatic GORD was not always present. Furthermore, as the GORD treatments varied between studies and were often of a short duration, it is hard to draw a common conclusion regarding their effects. However, for those individuals in whom GORD precipitates asthmatic episodes, treatment may be warranted and effective (Gibson et al, 2009).

COPD is a condition primarily caused by tobacco smoking characterised by airflow limitation that is not completely reversible (Celli et al, 2004). Anatomical changes in COPD including hyperinflation, elevated intra-abdominal and negative intra-thoracic pressures may predispose to relaxation of the lower oesophageal sphincter and reflux (Casanova et al, 2004). In a longitudinal cohort study, Garcia Rodriguez et al (2008) reported that a diagnosis of COPD results in a significantly elevated risk of a subsequent GORD diagnosis compared to a control group with no COPD. Furthermore, GORD may also contribute to acute exacerbations in COPD (Rascon-Aguilar et al, 2006).

Although the correlation between asthma, COPD and GORD is not clear, there are benefits associated with the treatment of GORD for respiratory conditions. Indeed, it has been reported that patients with both COPD and GORD have diminished quality of life compared to patients with COPD alone. Thus, by symptomatically managing GORD, it may be possible to improve quality life in COPD patients (Rascon-Aguilar et al, 2008). Furthermore, surgical correction of GORD results in a significant reduction in respiratory symptoms (Greason et al, 2002; Larrain et al, 1991). There have however been several reports that PPI therapy is associated with an increased risk of community-acquired pneumonia, especially in high-risk elderly patients so potential benefits should be weighed against risks in individual patients (Gulmez et al, 2007; Sarker et al, 2008; Eurich et al, 2010).

## Current Methods of GORD Diagnosis

The various methods employed currently in GORD diagnosis are: empiric proton pump inhibitor (PPI) therapy trial, oesophageal pH and multichannel intraluminal impedance monitoring, symptom questionnaires, endoscopy and barium swallow. The initial step in the diagnosis of GORD is an empiric PPI therapy trial. Symptom reduction whilst on PPI therapy establishes a diagnosis with a sensitivity of 75-78% and specificity of 80-90% when associated with an improvement in self-reported symptom scores •50% (Bautista et al, 2004; Dickman et al, 2005; Fass et al, 1998). However, a recent meta-analysis reported that short-term treatment with a PPI does not establish the diagnosis according to accepted reference standards. Furthermore, PPI therapy does not reduce the number of reflux episodes, only the acidity of the refluxate, such that non-acidic reflux episodes can still cause symptoms (Arevalo et al, 2011).

Oesophageal pH monitoring is the most broadly employed technique to monitor GORD. It is considered the gold standard in GORD diagnosis by providing data on both distal oesophageal acid exposure and its relation to symptoms. Percentage of the total time with pH < 4 is used as a measure of acid reflux (Dolder & Tutuian, 2010). Recent advances now allow direct insertion of a capsule into the oesophagus, as opposed to a nasooesophageal catheter, minimising patient discomfort (Dolder & Tutuian, 2010). However, 24-hour oesophageal pH monitoring is not sensitive enough to serve as a gold standard for GORD diagnosis. Reported sensitivities range from 79 to 96% and specificities from 85 to 100% (Behar et al, 1976; Stanciu et al, 1977; Johnsson et al, 1987; Rosen & Pope, 1989: Jamieson et al, 1992). Normal pH values are recorded in up to ¼ of subjects with oesophagitis and 1/3 of subjects with normal endoscopy findings (Dent et al, 1999). In addition, an important limitation of pH monitoring is that it cannot detect non-acidic reflux.

Oesophageal multichannel intraluminal impedance monitoring allows detection of both gaseous and liquid reflux. In combination with pH recordings, this modality has the capacity to identify all types of reflux (Roman et al, 2006). A combination of these techniques is emerging as the new gold standard in evaluating GORD (Dolder & Tutuian, 2010). However, limitations extend to the invasive, time-consuming and expensive nature of these tests, which prevents their large-scale use in the community.

Endoscopy and barium swallow are the best predictors of erosive oesophagitis and Barrett's oesophagus, which confirms a diagnosis of GORD. However, a large proportion of patients suffering from the cardinal GORD symptoms do

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# On the Scent of GORD Electronic Nose Profiling and Exhaled Breath Condensate Collection in the Diagnosis of Gastro-Oesophageal Reflux Disease

continued

not have oesophagitis, diminishing the sensitivity of this investigation (Galmiche et al, 1994). In addition, less than 20% of subjects with diagnosed oesophagitis complain of heartburn during episodes of reflux (Baldi et al, 1989). A demonstration of reflux during a barium study is not reliable in confirming a diagnosis, as this may be demonstrated in up to 20% of healthy controls (Fass et al, 2000). Such disadvantages in combination with the invasive and expensive nature of these modalities make them less desirable diagnostic tests for GORD diagnosis.

### Emerging Methods for the Diagnosis of GORD

Analysis of exhaled breath markers offers a rapid, non-invasive and reproducible approach to the diagnosis of GORD. Breath markers can be examined in the liquid phase (EBC) and the gaseous phase via volatile organic compound (VOC) analysis (electronic nose technology) and nitric oxide (NO).

### Nitric Oxide (NO)

Within the respiratory system, NO plays a vital role as a bronchodilator, vasodilator, nonadrenergic, non-cholinergic (NANC) neurotransmitter, and in addition is an inflammatory mediator (Yates, 2001). Derived from L-arginine in reactions catalysed by NO synthases, NO is released by eosinophils, macrophages and epithelial cells of the lung. The fraction of exhaled nitric oxide ($F_ENO$) can be measured rapidly and non-invasively, providing insight into airway inflammation and disease activity in numerous respiratory conditions (Kharitonov et al, 1994).

$F_ENO$ has been shown to be increased in asthmatic patients and can accurately discriminate between subjects with and without asthma (Heffler et al, 2006). In asthmap, measurement of $F_ENO$ has emerged as a promising method of monitoring disease activity and treatment response (ATS/ ERS, 2005, Horvath et al., 1998, Kharitonov et al., 1994, Sandrini, 2010). Furthermore, smaller but significant increases in $F_ENO$ have been demonstrated in subjects with uncontrolled COPD and ex-smokers with COPD compared to healthy controls and current smokers (Maziak et al, 1998; Montuschi et al, 2001).

### The Electronic Nose

Evidence exists for the use of olfaction in disease diagnosis in ancient civilisations from 2000 BC, with continued roles in modern medicine. Amongst others, the distinct odours associated with diabetic ketoacidosis, uraemia, and chronic liver failure are well documented. The electronic nose was initially designed to imitate the mammalian olfactory system. It is a chemical array sensor system for the identification of odours and volatile compounds consisting of a range of electronic chemical sensors. This allows the identification of a broad class of gases by individual sensors and the analysis of complex odours without identification of individual components (Gardner & Bartlett, 1994). Detection of volatile organic compounds (VOCs) leads to a modification of sensor properties including resistance and conductivity, which is then quantified. The unique response curve or "smellprint" generated is then evaluated by a pattern recognition system. After being trained with an adequate "training set", the e-nose can identify specific diseases based on the VOC pattern (Fend et al, 2006; Berna et al, 2009).

Electronic nose technology has applications and practicality in medicine in that it is portable, non-invasive and rapid. Too date, it has showed promise in distinguishing controls from subjects with pneumonia (Hockstein et al, 2004), distinguishing cerebrospinal fluid from serum (Thaler et al, 2000), identifying organisms responsible for urinary tract infections (Pavlou et al, 2002) and diagnosing diabetes (Ping et al, 1997). Further applications of this technology extend to food quality assessment, environmental monitoring and the detection of noxious gases in aeronautics (Gouma & Sberveglieri, 2004; Mahmoudi, 2009; Röck et al, 2007).

In the field of respiratory medicine, electronic nose technology has several potential applications. Exhaled breath contains over 3000 identifiable VOCs (Yates et al, 2011). It distinguishes asthmatic patients from controls, with an increased performance when combined with FE¬NO (Montusci et al, 2010), as well as those with non-small cell lung cancer from both COPD and healthy control groups (Dragonieri et al, 2009). Because the breath profiles of asthmatic and COPD patients can be distinguished with an accuracy of 96% ($p < 0.0001$), this technology may ave application in distinguishing between obstructive airway conditions (Fens et al, 2009) (Figure 3). Whilst there is only early research examining the use of electronic nose technology in distinguishing GORD subjects from other groups (Timms et al, 2011), existing data on profiles in lung disease provides a strong base from which an evaluation of GORD as a single entity and in lung disease can be performed. Electronic noses have the potential to not only detect pepsin, but also to add hydrogen ion and bile acids in a multi-dimensional method of detecting GORD.

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# On the Scent of GORD Electronic Nose Profiling and Exhaled Breath Condensate Collection in the Diagnosis of Gastro-Oesophageal Reflux Disease

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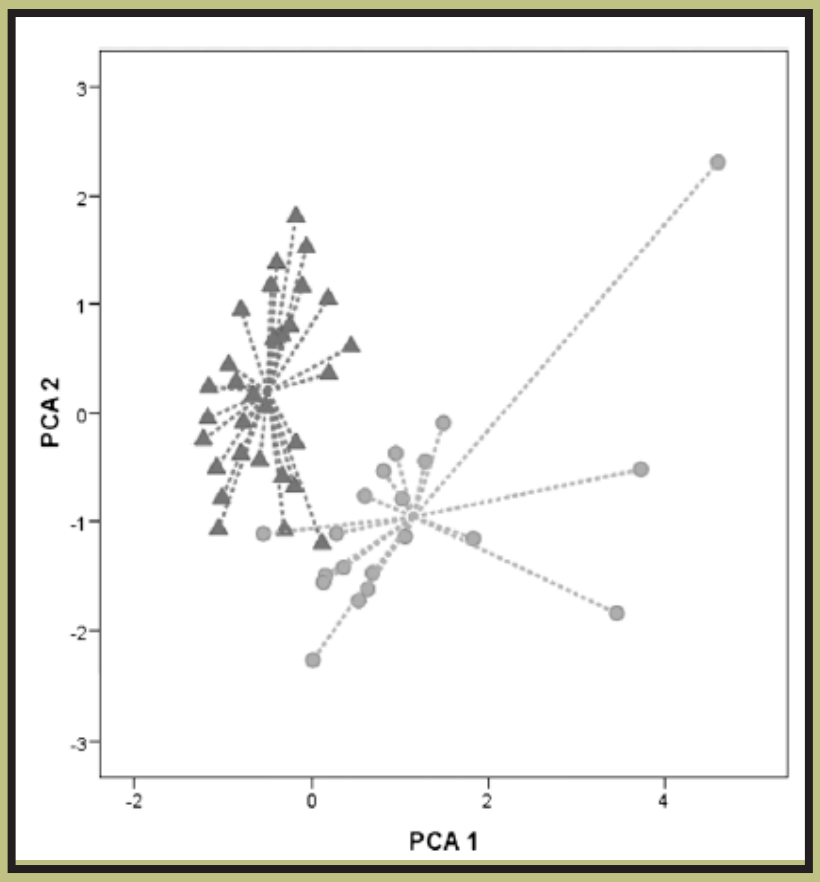


Figure 3: *Two-dimensional principal component analysis (PCA) plot, which displays a differentiation between subjects with COPD (triangles) and asthmatic subjects (circles) with an accuracy of 96%; p < 0.0001 (From Fens et al, 2009).*

## Exhaled Breath Condensate

Collection and analysis of EBC is a simple and non-invasive means of sampling the lower respiratory tract. The respiratory lining fluid contains various non-volatile and volatile substances, which can be sampled using EBC (Yates et al, 2011). The main component of EBC is condensed water vapour (Effros et al, 2002), however, EBC also contains respiratory droplets with non-volatile compounds including oxides of nitrogen, cytokines, oxidation products and hydrogen peroxide and readily quantifiable pH, which may be useful in disease detection (Horvath et al, 2005).

For sample collection, subjects are instructed to breathe tidally into a mouthpiece for 10 minutes in order to collect approximately 1-3mL of EBC. The high water vapour content allows condensation by passing breath through a cooling device. There are a number of issues that exist with the collection of EBC, which is a novel biological fluid about which much needs to be learned. Firstly, although EBC samples the entire respiratory system from mouth to alveoli, the contribution of each part to EBC is still unclear (Jackson et al, 2007). In addition, the composition of the material used to collect EBC can alter the composition of the condensate collected such that different devices are not directly comparable (Liu et al, 2007). Despite these limitations, EBC is gaining increasing popularity as a research tool in conditions including cystic fibrosis, asthma, COPD, bronchiectasis and idiopathic pulmonary fibrosis (Hunt, 2002).

### pH

One such application involves the measurement of pH in EBC. After de-aeration with CO2 free gas, EBC pH measurements are robust and highly reproducible over long periods of time (Accordino et al, 2008; Vaughan et al, 2003). Combined with evidence that EBC pH is significantly reduced after administration of pharyngeal acid, such a method may have relevance in the diagnosis of GORD (Hunt et al, 2006). EBC pH has been reported as being lower in subjects with GORD symptoms and asthma. In a study of stable asthmatic subjects, EBC pH was not lower overall, however, there was a cluster of subjects with pH < 6.5 and a significant association with GORD symptoms (Liu et al, 2011). In a study of COPD patients with exacerbations, EBC pH was found to be significantly lower in subjects with GORD symptoms when compared to COPD subjects with no GORD symptoms (pH 6.47, 7.17 respectively, p=0.02). pH was also lower in control subjects with GORD symptoms (pH=6.34) when compared to control subjects without GORD symptoms (pH 6.34, 7.22 respectively, p=0.03). However, one limitation of this study was the lack of 24-hour pH monitoring and a reliance on GORD symptom questionnaires (Terada et al, 2008).

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# On the Scent of GORD Electronic Nose Profiling and Exhaled Breath Condensate Collection in the Diagnosis of Gastro-Oesophageal Reflux Disease

continued

| Study Population | Methods | Results |
|---|---|---|
| Adults with chronic rhinosinusitis (n=33) (Ozmen et al (2008) | Measurement of pepsin in nasal secretions via nasal lavage and pharyngeal pH monitoring. Pepsin activity determined by a modified version of a proteolytic enzyme assay. | Pepsin activity was detected in 82% of subjects in the study group and 50% of the control group (p=0.014). All subjects with positive pepsin assays had laryngopharyngeal reflux according to pharyngeal pH monitoring. When compared to pH monitoring, the assay was 100% sensitive and 92.5% specific. |
| Children (n=96) with a range of respiratory diseases (Starsota et al, 2007). | BAL fluid pepsin concentration determined using a modified version of the proteolytic enzyme assay method. This was compared to a reflux index calculated as percentage of measurement time with oesophageal pH < 4 during 24 hours. | Correlation between the number of reflux episodes and concentration of pepsin in BAL fluid (p< 0.0001). Results may be confounded by assay interference with other acidic proteases in the lung and pulmonary pepsinogen. |
| Adults with laryngopharyngeal reflux (n=23) (Knight et al, 2005). | Subjects with laryngopharyngeal reflux provided throat sputum samples whilst undergoing pH monitoring. Sputum samples were assayed for pepsin using ELISA with goat antibodies. | The assay was 100% sensitive and 89% specific. Pepsin was detected in patients without a reflux event as determined by pH; thus, it is plausible that pepsin may be a more appropriate marker for laryngopharyngeal reflux. |
| Stable lung transplant population (n=13) (Ward et al, 2005) | BAL fluid supernatants were assessed for pepsin using ELISA with mono-specific antibody to porcine pepsin (lower limit of detection <1ng/ml). Pepsin levels were compared to healthy controls (n=4). | Pepsin activity was present in all BAL samples from transplant recipients with levels below limit of detection in the control group (p< 0.003). |
| Suctioned tracheal secretions were obtained from mechanically ventilated patients (n=30) (Metheny et al, 2002). | Tracheobronchial secretions were assessed using ELISA with rooster polyclonal antibodies to human pepsin (lower limit of detection < 1µg/mL). | Fourteen specimens from 5 patients returned positive for pepsin with pepsin associated with a flat position of the bed head (p<0.01). |

Table 1: *A summary of current studies examining pepsin detection in relation to ear, nose, throat, respiratory conditions and GORD.*

### Pepsin

Pepsin is a proteolytic enzyme produced by the chief cells of the gastric fundus. It is secreted as the zymogen pepsinogen before cleavage into its active form by the acidic nature of the stomach (Samuels et al, 2010). Pepsin is highly active at pH 2.0, but can also elicit tissue damage in the respiratory system at higher pH values, and is not fully inactivated until the pH reaches 6.5 or greater (Johnston et al, 2003). The composition of gastro-oesophageal refluxate is variable in that it may contain acid or bile salts. However, pepsin is always present, thus, the presence of pepsin in airway samples provides evidence for gastric reflux, but will not detect reflux that does not result in aspiration (Potluri et al, 2003).

The detection of pepsin is becoming increasingly prevalent in relation to GORD in the ear, nose and throat literature, as summarised in Table 1. In addition, pepsin and bile acids have been isolated from the lower airways using bronchoalveolar lavage (BAL) in lung transplant patients with GORD (Gagermeier et al, 2010). Furthermore, Strugala et al (2007) have recently reported the detection of pepsin in EBC of chronic cough patients and pepsin has also been detected in the EBC of lung transplant patients with GORD (Krishnan et al, 2007).

Study Population Methods Results

Adults with chronic rhinosinusitis (n=33) (Ozmen et al (2008) Measurement of pepsin in nasal secretions via nasal lavage and pharyngeal pH monitoring. Pepsin activity determined by a modified version of a proteolytic enzyme assay. Pepsin activity was detected in 82% of subjects in the study group and 50% of the control group (p=0.014). All subjects

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with positive pepsin assays had laryngopharyngeal reflux according to pharyngeal pH monitoring. When compared to pH monitoring, the assay was 100% sensitive and 92.5% specific.

Children (n=96) with a range of respiratory diseases (Starsota et al, 2007). BAL fluid pepsin concentration determined using a modified version of the proteolytic enzyme assay method. This was compared to a reflux index calculated as percentage of measurement time with oesophageal pH < 4 during 24 hours. Correlation between the number of reflux episodes and concentration of pepsin in BAL fluid ($p< 0.0001$). Results may be confounded by assay interference with other acidic proteases in the lung and pulmonary pepsinogen.

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Stable lung transplant population (n=13) (Ward et al, 2005) BAL fluid supernatants were assessed for pepsin using ELISA with mono-specific antibody to porcine pepsin (lower limit of detection <1ng/ml). Pepsin levels were compared to healthy controls (n=4). Pepsin activity was present in all BAL samples from transplant recipients with levels below limit of detection in the control group ($p< 0.003$).

Suctioned tracheal secretions were obtained from mechanically ventilated patients (n=30) (Metheny et al, 2002). Tracheobronchial secretions were assessed using ELISA with rooster polyclonal antibodies to human pepsin (lower limit of detection < 1µg/mL). Fourteen specimens from 5 patients returned positive for pepsin with pepsin associated with a flat position of the bed head ($p<0.01$).

The clinical significance of pepsin detection in the respiratory tract is not yet clear. As the relationship between GORD and respiratory conditions is not well defined, it is difficult to ascertain whether treatment will affect clinical outcomes. Small volumes of gastric content are prevalent in the lungs of healthy subjects, with no detectable deleterious impacts on the respiratory system (Bartlett & Gorbach, 1975). This may impact on the reliability of this method, and suggests the need for further research to correlate pepsin concentration with reported GORD symptoms and respiratory symptoms in order to ascertain any significance.

## Conclusions

Whilst early evidence seems promising, further research is needed to investigate the potential of EBC measurements of pepsin and pH as well as the use of electronic nose technology in the diagnosis of GORD. Pepsin detection overcomes many of the issues associated with pH including the detection of non-acidic reflux in a non-invasive manner. Electronic noses have the potential to detect GORD in a multi-dimensional method encompassing pepsin, hydrogen ions and bile acids and may play a role in the future diagnosis of GORD.

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# AACSS 2011: Abstracts

Ascension Wines, Matakana, New Zealand
7-9 December 2011

*Conference Organiser* Christine Lamont
*Organising Committee* Richard Newcomb, John Ingram, Jeremy McRae, Sara Jaeger

## PLENARY LECTURE I

### MOLECULAR AND CELLULAR BASIS OF BITTER TASTE

**Wolfgang Meyerhof**

*German Institute of Human Nutrition Potsdam-Rehbruecke, Department of Molecular Genetics, Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Germany*

Bitter taste involves the recognition of countless molecules with different chemical structures. Depending on dose, these molecules can be toxic or healthy. Accordingly, consumers strongly prefer some bitter tasting foods and beverages while they avoid others.

For a detailed understanding of bitter taste physiology and its importance for food selection we first uncovered the response profiles of TAS2R bitter taste receptors of various species to numerous bitter chemicals and elucidated the principles of ligand-TAS2R interactions. We also examined, in man and mice, the TAS2R repertoire of the oral bitter-responsive receptor cells. Moreover, we investigated mice with genetically modified TAS2R alleles by means of molecular biological, electrophysiological and behavioral methods. Finally, we examined the connection of oral bitter receptor cells with the first order gustatory brain stem neurons by monitoring induction of a neural excitation marker.

The results reveal subpopulations of bitter-dedicated oral sensor cells that detect different sets of bitter compounds and are differently connected to 1st order central neurons. The implications of these findings for bitter compound avoidance will be discussed.

### INDUCTION OF BITTER TASTE SIGNALLING, BY PLANT COMPOUNDS, IN THE ENTEROENDOCRINE CELL LINE, HUTU-80

**Edward Walker** and John Ingram

*The New Zealand Institute of Plant & Food Research Limited*

Chemosensory systems in the gastrointestinal (GI) tract play an important role in monitoring the composition of gut luminal contents during digestion and subsequent modulation of gut function and feeding behaviour. Bitter food components have been implicated in the post-ingestive modulation of satiety and represent a possible mechanism for the development of foods enhanced with appetite control functionality. Mucosal enteroendocrine cells detect these bitter compounds through bitter taste receptors (TAS2Rs), which signal via intracellular calcium to release appetite-regulating peptide hormones such as CCK. We are examining the effect of known bitter-tasting compounds, phytochemical extracts, and pure phenolic standards on calcium signalling in the human enteroendocrine cell line HuTu-80. Intracellular calcium release was quantified in these cells using the calcium-sensitive dye Fluo-4 AM and various anion channel blockers (probenecid, sulfinpyrazone) that block dye leakage. Known bitter compounds, such as denatonium benzoate, chlorhexidine and chloramphenicol, induce calcium signalling in HuTu-80 cells. We then went onto screen a series of phytochemical extracts for their ability to induce bitter taste signalling in these cells, and found one extract in particular that was a highly potent inducer of calcium release. These data suggest that HuTu-80 cells may be a useful screening tool for the identification of bitter compounds and plant extracts that may have application in modulating appetite.

### SIMILARITY OF THE PORCINE AND HUMAN SWEET TASTES (NEW RESULTS IN PIGS)

B. Shrestha[1], M. Swart[2], S. Osuna[3], Y. Zeng[1], M. Larequie[1] and **E. Roura*[1]**

[1]*Centre for Nutrition and Food Sciences (QAAFI), The University of Queensland, Australia*

[2]*Institut de Química Computacional and Departament de Química and Institució Catalana de Recerca i Estudis Avançats (ICREA), Universitat de Girona, Spain*

[3]*Department of Chemistry and Biochemistry, University of California, Los Angeles, USA*

**Presenting Author*

Electrophysiological recordings from pig tongue show that sugars are sensed to a similar extent to that observed in humans. On the contrary, none of the high intensity sweeteners (HIS) tested to date in pigs (e.g. saccharin, stevioside and thaumatin among others) gave taste responses close to those in humans. The electrophysiological technique is valuable and accurate to measure generic taste activity in mammals but is invasive and complex. Double Choice (DC) models using water solutions have been widely used in laboratory rodents and showed potential results in pigs. The aim of this project was to study dose related sweetener preferences in pigs and compare them to humans. We tested sugar (control) and some of the HIS known in humans (saccharin, stevioside, rebaudioside-A, acesulfame-k and sucralose). We used an adaptation of a DC model based on water solutions. Our results confirmed that pigs, as expected, perceive sugar but not saccharin very similar to humans. In general, none of the HIS tested were perceived in pigs as intensely as in humans. However, the high responses recorded for rebaudioside-A and sucralose (high) were closer to the human thresholds than other HIS (low). A structure activity function (SAF) model of the human sweet taste receptor hT1R2 recently developed by our group shows an inner and an outer binding site for agonists. How our hT1R2 SAF model may help explain the differential interactions of the two groups of HIS in pigs (high vs. low) will be discussed.

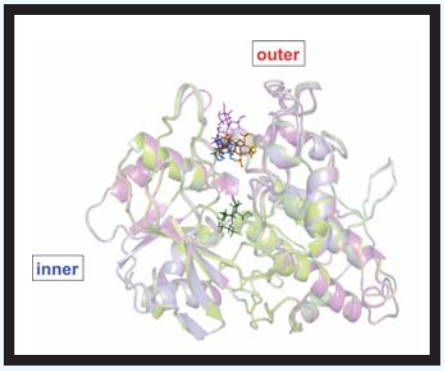


Figure 1. *Representation of the inner and outer binding site.*

### CHARACTERISATION OF A NATURALLY OCCURRING OLFACTORY RECEPTOR VARIANT IN *DROSOPHILA MELANOGASTER*: IMPLICATIONS FOR LIGAND BINDING

Marien de Bruyne, Katherine Shaw and **Coral Warr**

*School of Biological Sciences, Monash University, Clayton VIC 3800, Australia*

Insect olfactory receptor *(Or)* genes are large, rapidly evolving gene families that are of considerable interest for evolutionary studies as they determine the responses of sensory neurons which mediate critical behaviours and ecological adaptations. One member of the *Or* gene family, *Or22a*, shows multiple duplication events across *Drosophila* species, and there are dramatic changes in the response profile of the olfactory receptor neuron class in which *Or22a* is expressed,

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called ab3A (1). This contrasts with most neuron classes for which response properties are generally well conserved across **Drosophila** species, suggesting that the changes in response of the ab3A neuron may be important in ecological adaptation. In our standard laboratory strain of *D.melanogaster*, Canton-S, two closely linked and highly homologous genes, *Or22a* and *Or22b*, are thought to be expressed in ab3A neurons but only *Or22a* mediates the ab3A response (2). Interestingly, at this locus high levels of allelic differentiation were found in natural populations along the east coast of Australia (3). The major source of this variation is a deletion mutation that creates a fusion of the Or22a and *Or22b* genes, *Or22del*, predicted to encode a single functional chimaeric receptor. The allele frequency of this deletion mutation clines along the east coast, with high frequency in northern populations suggesting it is under positive selection. We have shown that in flies homozygous for the *Or22del* allele there are significant changes in the response profiles of the ab3A neurons compared to flies that do not contain the deletion. This natural variant provides an opportunity to identify ligand-binding domains/residues in *Or22a* by comparing the responses and sequences of the *Or22a* and *Or22del* receptors.

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## FRET REVEALS NOVEL INTERACTIONS BETWEEN INSECT OLFACTORY MEMBRANE PROTEINS

**Pablo Germán[1,2]**, Seléne van der Poel[1], Colm Carraher[1,2], Kai Xiong[2], Andrew V. Kralicek[1], Richard D. Newcomb[1].

[1] *The New Zealand Institute of Plant & Food Research Limited*

[2] *School of Biological Sciences, University of Auckland*

Insect odorant receptors (ORs) are a novel family of ligand-gated cation channels that can respond to volatile organic compounds at low concentrations. They are involved in the detection of odorants implicated in mate recognition, food detection and predator warning. These receptors form a complex containing at least two subunit members; the noncanonical Orco and a ligand-binding receptor subunit. As well as these receptors subunits, the integral membrane proteins SNMP1 and SNMP2 are also associated with olfactory function, with SNMP1 required for pheromone reception in *Drosophila melanogaster*. We have used FRET in live insect cells to investigate protein-protein interactions among these membrane proteins. Using baculovirus-mediated expression to produce proteins fused with CFP or YFP we were generally able to produce full length fusion proteins, although much of the recombinant protein was located intracellularly. Intermolecular FRET efficiencies between different pairs provide evidence of interaction between the ligand binding OR, Or22a, and Orco, as well as homomeric interaction for both subunits. The other integral membrane protein family member SNMP1 interacted with Or22a but not Orco, suggesting an additional role for SNMP1 in odorant reception, while SNMP2 did not interact with either Orco or Or22a. Together these data suggest a new stoichiometry for the olfactory receptor complex and a possible new role for SNMP1.

## BIOCHEMICAL CHARACTERISATION OF THE INSECT OLFACTORY COMPLEX

**Colm Carraher[1],** Andrew Kralicek[1], David Christie[2], Richard Newcomb[1].

[1] *The New Zealand Institute of Plant & Food Research Limited*

[2] *School of Biological Sciences – University of Auckland*

Insect odorant receptors (ORs) are members of a novel family of seven-transmembrane ion channels. There is also evidence from some in vitro studies in HEK293 cells that they may also be able to signal via G protein pathways. One highly conserved insect OR, Orco, is essential for these activities, potentially forming a complex with ligand-binding ORs.

Many questions remain concerning the structure and function of insect ORs including: What is the minimum number of receptor units required to form a functional channel? What is the stoichiometry of the Or83b/ligand-binding OR complex? Where do ligands bind in the ligand-binding ORs? What components of the complex and additional factors are required for ionotropic and metabotropic signalling?

Biochemical approaches that we are employing to explore many of these questions will be discussed, including heterologous cell and cell-free expression, co-purification studies, antibody pull down assays, ligand binding assays and studies in artificial membranes and proteoliposomes. Purification of the receptors is essential for binding studies using SPR and eventually crystallography. The steps towards the purification of these membrane-bound proteins will be outlined.

These studies will increase our understanding of the structure/function of the insect olfactory complex, and pave the way to their use in odour sensing devices.

## OLFACTORY SENSILLA AND ELECTROPHYSIOLOGICAL RESPONSES OF THE ANTENNA TO HOST ASSOCIATED ODOURS OF THE BLOWFLY *CALLIPHORA STYGIA*

**Olivia Leitch[1,2*],** Chris Lennard[1], Paul Kirkbride[3], James Wallman[4], and Alisha Anderson[2]

[1] *National Centre for Forensic Studies, Faculty of Applied Science, University of Canberra, Canberra, ACT, Australia*

[2] *CSIRO Division of Ecosystem Sciences and Food Futures Flagship, Canberra, ACT, Australia*

[3] *Forensic and Data Centres, Australian Federal Police, Canberra, ACT, Australia;*

[4] *Institute for Conservation Biology and Environmental Management, School of Biological Sciences, University of Wollongong, NSW, Australia.*

*Calliphora stygia*, an Australian native blowfly, is primarily carrion-dependant with respect to its feeding and reproductive behaviour. This species is consistently encountered during the early stages of decay and is of significance in forensic entomology for estimations of time since death. It is known anecdotally that carrion blowflies identify and locate cadavers via volatile organic compounds (odours). However, little research has been conducted on blowfly olfaction, particularly with respect to the specific odorants attracting forensically important carrion flies to a host source. The aim of this research was to examine the morphological features of the olfactory organs of *C. stygia* and the antennal electrophysiological response of this species to a range of volatile compounds associated with human decomposition. The morphology of the antennae and maxillary palps, along with their associated sensilla, were studied via scanning electron microscopy. On each antenna, three distinct types of sensilla were identified in both sexes: 1) sensilla (s) trichodea; 2) s. basiconica (subtypes large, thin and small); and 3) s. coeloconica. Sexual dimorphism was detected in the relative expression levels of s. trichodea, which were greater in female flies, while male flies had more s. basiconica. No significant difference between the sexes was observed in overall antenna length and width. Besides mechanoreceptors, only s. basiconica (subtypes large and small) were observed on the maxillary palps. Electroantennagraphy analyses assessing the olfactory response of the third antennal segment show that *C. stygia* respond physiologically to a variety of chemically diverse odorants associated with human decomposition.

## FUNCTIONAL ANALYSIS OF NEMATODE GPCRS IN YEAST

**Muhammad Tehseen,** Alisha Anderson, Mira Dumancic and Stephen Trowell

*CSIRO Food Futures Flagship and Division of Ecosystem Sciences, Black Mountain Laboratories, Canberra, Australia*

Given its ease of genetic manipulation, rapid growth, and eukaryotic secretory pathway, yeast is an attractive host system for the development of robust heterologous expression systems. Yeast has been successfully used for the heterologous expression of mammalian membrane proteins, specifically G-protein coupled receptors (GPCRs). Although the *Caenorhabditis elegans* genome was sequenced 13 years ago and encodes over 1,000 GPCRs, of which several hundred are believed to respond to volatile organic ligands, only one of these receptors, ODR-10, has been linked to a specific ligand, 2,3-butanedione. Here we report the tailoring of a *Saccharomyces cerevisiae* strain for the analysis of *C. elegans* olfactory receptor function. In this study, a yeast *gpa1Δ ste2Δ* double mutant was used to develop a strain that efficiently couples a nematode olfactory receptor with the yeast signalling pathway. We used three different reporter genes: green fluorescent protein (GFP), histidine ($HIS_3$) and MEL1 to verify activation of the signal transduction pathway by ligand-GPCR interactions. With this heterologously engineered yeast system, we will be able to accelerate the de-orphaning of *C. elegans* GPCR proteins.

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## OLFACTORY ENSHEATHING CELLS: HOW DIFFERENT SUBPOPULATIONS REGULATE AXON GUIDANCE

**James St John** and Jenny Ekberg

*Eskitis Institute for Cell and Molecular Therapies, Griffith University, Nathan 4111, Brisbane, Queensland, Australia, j.stjohn@griffith.edu.au*

The glia of the olfactory system, olfactory ensheathing cells (OECs) are intimately associated with the axons of primary olfactory neurons that extend from the olfactory epithelium to their targets within the olfactory bulb. However, OECs are not a uniform population but instead there are different subpopulations each with a different molecular profile and proposed role in vivo. We have used OEC-axon assays and determined that OECs play an active role in modulating the growth of pioneer olfactory axons. The motility of OECs was mediated by GDNF, which stimulated cell migration and increased the apparent motility of the axons whereas loss of OECs via laser ablation of the cells inhibited olfactory axon outgrowth. These results demonstrate that the migration of OECs strongly regulates the motility of axons and that stimulation of OEC motility enhances axon extension and growth cone activity. We then determined that axons respond differently to OECs derived from the peripheral region of the olfactory nerve or from the olfactory bulb. We purified OECs from anatomically distinct regions of the olfactory bulb and used cell behaviour assays to reveal that OECs from the olfactory bulb are a functionally heterogeneous population with distinct differences which are consistent with their proposed roles in vivo. These results demonstrate that OECs from the olfactory bulb are a heterogeneous population that use lamellipodial waves to regulate cell-cell recognition.

## UNDERSTANDING THE PATHOPHYSIOLOGY OF MELIOIDOSIS: THE NASAL EPITHELIUM AND OLFACTORY NERVE CONSTITUTE A PATHWAY FOR BACTERIAL INVASION OF THE BRAIN

**Jenny Ekberg[1],** Ifor Beacham[2], Alan Mackay-Sim[1] and James St John[1]

[1] *Eskitis Institute for Cell and Molecular Therapies, Griffith University, Brisbane, Australia;*

[2] *Institute for Glycomics, Griffith University, Gold Coast, Australia*

Melioidosis is a potentially fatal disease which is caused by the bacteria *Burkholderia pseudomallei.* The disease is endemic to northern Australia with an incidence of 15.5/100,000 amongst the general population, 42/100,000 in the Indigenous population and a **mortality rate of 14%.**

We have recently discovered that a minor infection affecting as few as 20-50 cells of the olfactory epithelium lining the nasal cavity is sufficient to initiate a cascade of events that lead to infection of the brain within 24-48 hours. Using a mouse model, we have determined that the bacteria penetrate the basal layer of the olfactory epithelium and enter the bundles of axons that form the olfactory nerve. Once the bacteria enter the bundles of axons, the nerve cells die and the supporting cells, olfactory ensheathing glial cells, react by dramatically altering their morphology to form open tube-like structures. Thus, the olfactory nerve bundles become open conduits that extend from the nasal epithelium right into the brain. The bacteria then proliferate and migrate to enter and infect the brain. Importantly, we have previously determined that the cells of the immune system, macrophages, are largely excluded from olfactory nerve bundles and thus the bacteria within the nerve bundles are fairly isolated from the body's natural defense mechanisms.

It is likely that the mechanism of infection along the olfactory nerve is common to other bacteria and viruses and thus the results from the project will provide greater understanding of the impact of nasal infections and will facilitate the design of new treatments.

## MYCBP2 CONTROLS GUIDANCE OF ROBO2 AXONS DURING DEVELOPMENT OF THE MURINE OLFACTORY SYSTEM

Mr Gregory James[1], Julie Conway[1], Timothy O' Brien[2], Prof Brian Key[1], **Dr Annemiek Beverdam[1]**

[1] *School of Biomedical Science, University of Queensland*

[2] *Department of Biomedical Sciences, Cornell University, Ithaca, New York. USA*

ROBO and SLIT molecules are crucial axon guidance factors during the development and regeneration of the central nervous system. However, the molecular interactions that these molecules take part in to shape the axon tracts in the CNS remain largely unknown. MYCBP2 is a strongly conserved E3-ubiquitin ligase that regulates axon and synapse development through interactions with multiple signaling pathways. Here we describe how a subpopulation of ROBO2 expressing olfactory sensory neurons is severely misguided along the dorsoventral axis of the olfactory bulbs in *Mycbp2* loss of function mice, in a pattern strikingly reminiscent of that described for *Slit1* and *Robo2* mutant mice. We observed a significant loss of innervation in a large dorsal domain in the olfactory bulb. In addition, we showed that these dorsal ROBO2 expressing neurons do not die, but instead appear to stall in the ventral outer nerve layer, where they fail to refasciculate with homotypic dorsally targeting axons. In addition, we uncovered strong genetic interactions between *Mycbp2* and *Robo2* in double mutant mice. Altogether, these data suggest that Mycbp2 controls the guidance of ROBO2 expressing neurons during development of the olfactory system and provide important new insights into the genetic cascades that regulate axon guidance processes in the CNS controlled by classic guidance factors ROBO and SLIT.

## OEC PROLIFERATION IN EMBRYONIC MICE OLFACTORY BULB

**Daniel A. Amaya,** Jenny A.K. Ekberg, Fatemeh Chehrehasa, James A. St John, Alan Mackay-Sim

*National Centre for Adult Stem Cell Research, Eskitis Institute for Cell and Molecular Therapies, Griffith University, Nathan, Brisbane 4111 Queensland, Australia*

In recent years the growing interest in the olfactory system has grown significantly. This can be attributed to the unique environment formed by the residing cells of the olfactory system. Most import is the glia of the olfactory system, named Olfactory Ensheathing Cells (OECs). OECs have shown remarkable capabilities in the mechanisms behind neural regeneration. They have shown to be essential in the regrowth of damaged axons, whether by normal turnover or after an injury. With the aid of our OMP-ZsGreen x S100-DsRed transgenic mice that allow us to visualise olfactory neurons and OECs, we are able to study the olfactory system in great detail. Here we are using Ethynyl deoxyuridine (EdU) to identify the proliferating niche of OECs during early development. We have identified that between E13 and E17 (repeats, n=3) the population of OECs in the OB are at the peak of proliferation and migration. We have shown how proliferating OECs migrate into the Nerve Fibre Layer (NFL) of the Olfactory Bulb and form a distinction between the inner NFL and the outer NFL. Double-labelling of OECs was used to aid in their identification and to distinguish them from proliferating macrophages. We used a one-way ANOVA on the data obtained from the experiment which supported our findings.

## METHODOLOGY FOR RAPID SENSORY SCREENING OF A WIDE NUMBER OF HOP SAMPLES FOR A SPECIFIC AROMA TRAIT

**B. Pineau**, A. Paisley, M.W. Wohlers, S.R. Jaeger

*The New Zealand Institute for Plant and Food Research Limited, New Zealand*

*pineau.benedicte@plantandfood.co.nz*

Aroma-type hops are essential components of the brewing process imparting many of the specific flavours and aromas that define the style of beer. Hop breeding research aims to develop selections that would confer new, unique sensory characteristics to beer.

The identification of promising seedling plants relies primarily on chemical analysis of secondary plant metabolites, because collecting sensory data on breeding populations of several hundreds of individual plants is a significant challenge. This challenge was addressed by developing a sensory methodology based on a unique experimental design to rapidly screen a large number of dried hop samples for a specific aroma trait. Hop samples (n=210) were assessed for the perceived intensity of citrus aroma by a 6-member trained sensory panel. The sensory screening data were collected over seven days. On each day, each panellist assessed a total of 54 samples that comprised of 3 control samples presented six times and 36 unique samples presented once, 6 of which were carried forward to the day after for assessment replication. The control samples were chosen for exhibiting weak, moderate and strong intensities of citrus aroma, respectively.

The quality of the screening data collected was estimated through series of analyses of variance. On each day, the panel was found to differentiate significantly between the three control samples, which showed the expected weak, moderate and strong intensity scores for citrus aroma. Also, the mean sensory scores of the 42 samples carried forward over two consecutive days were not found to significantly differ between the two assessment replications (t-test: $t_{d.f.=502} = -1.38$, $P = 0.17$).

Overall, the methodology developed was shown to

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provide robust sensory data and could potentially be applied to other food-related products. As an example, it might be of interest to identify rapidly tea leaves exhibiting unique aroma characteristics.

### SOURCE OF ORIGIN DIFFERENCES IN SAUVIGNON BLANC WINES: SENSORY AND CHEMICAL ANALYSES

**[1]Wendy Parr,** [2]James Green, [1]Jason Breitmeyer, [3]Dominique Valentin, and [1]Robert Sherlock

[1] *Lincoln University, Christchurch, New Zealand;*

[2] *University of Otago, Dunedin, New Zealand;*

[3] *CSGA Centre des Sciences du Goût et de l'Alimentation, UMR-6265 CNRS, UMR-1324 INRA, Université de Bourgogne, AgroSup Dijon, France.*
*E-mail : parrw@lincoln.ac.nz*

*Key words: Sensory; chemical composition; Sauvignon*

In the present study, Sauvignon blanc wines of New Zealand (Marlborough), French (Sancerre; Loire; Saint Bris), and Austrian (Styria) origin were investigated to determine differences in perceived flavour profile and in chemical composition as a function of country of origin. For the sensory investigation, 19 New Zealand wine professionals evaluated 18 Sauvignon wines, 6 from each source of origin. Sensory methods included intensity ratings to experimenter-provided descriptors, typicality ratings, and classification tasks (non-directed and directed sorting). Results demonstrated that wines from the three sources of origin were discriminated as different, with New Zealand wines dominated by perceived green characteristics, Austrian wines perceived to be fruity (stone-fruit), and French wines relatively subdued in all characteristics measured other than perceived minerality. The chemical analysis investigated chemical compounds considered important to Sauvignon blanc wine sensory properties. This included determination of concentrations of fermentation-derived, volatile aroma compounds (including acetate esters, fatty acid ethyl esters, higher alcohols and volatile acids) and isobutylmethoxypyrazine using the automated HS-SPME (Headspace Solid-Phase Micro-Extraction) technique [1] and a modification of the HS-SPME technique [2]. Analysis of thiol concentrations was undertaken by SPME-GC-MS/MS analysis. The sensory and chemical data were associated statistically, demonstrating that the chemical compounds clustered into three groups, each cluster associated with one source-of-origin. A 'green' cluster of compounds (e.g., hexyl acetate; trans-3-hexen-1-ol) associated closely with the New Zealand wines, a 'ripe/fruity' cluster of compounds (e.g., ethyl acetate; ethyl butanoate; isoamyl acetate) associated with Austrian wines, and other compounds (e.g., ethyl butyrate; benzaldehyde) associated with French wines. In conclusion, the data demonstrate differences in perceived sensory characteristics and chemical composition of Sauvignon wines as a function of wine source-of-origin, along with relations between specific aromatic chemical compounds and sensory terms employed by wine professionals.

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### ODOUR SENSITIVITY IN HUMANS: IMPACT ON FLAVOUR PERCEPTION AND ACCEPTABILITY

**FR Harker** and SR Jaeger

*The New Zealand Institute for Plant & Food Research Ltd, Auckland, New Zealand*

Flavour perception is idiosyncratic. In many instances, the way a flavour is perceived and the extent to which it is liked/disliked varies from person to person. We illustrate such differences using examples from 10 odorants common to a range of foods and beverages, and also discuss if some people generally are more odour sensitive than others. Examples of how varying odour acuity can impact food acceptability and selection are presented (linked to cis-3-hexen-1-ol and b-ionone). In the closing part of the presentation, we highlight challenges facing researchers trying to link odour sensitivity to food acceptability/selection and note than food-related behaviour is influenced by factors other than odour acuity. Non-sensory factors (eg, habit, culture) may override sensory impact.

### WOULD A ROSE SMELL AS SWEET TO EVERYONE?

**Jeremy McRae[a],** Kelly Atkinson[a], Lauren Axten[a], Christina Bava[a], Michelle Beresford[a], Sok Leang Chheang[a], Jo Gamble[a], Amy Paisley[a], Mei Peng[a], Benedicte Pineau[a], Helene Reinbach[ab], Simon Rouse[a], Leah Tooman[a], Jana Watt[a], Mark Wohlers[a], Sara Jaeger[a], Richard Newcomb[ac]

[a], *The New Zealand Institute for Plant & Food Research Ltd*

[b], *University of Copenhagen, Denmark,*

[c] *School of Biological Sciences, University of Auckland*

Humans have an incredible array of foods available for consumption. Cultural and personal influences affect our choice of foods to eat. We hypothesize that the ability to smell also plays an important role in determining food preferences. The ability to detect certain odors can vary considerably among individuals. We determined the ability of 150 individuals to detect ten odorants found in foods and beverages and searched for the genetic bases of these abilities using a genome-wide association approach. We have identified that sensory acuity to three out of the ten odorants are, in part, genetically determined. The regions that we identified as being associated with the ability to detect these three compounds were all located near, or within, clusters of odorant receptor genes, and explain a large proportion of the variance in the ability to detect these odorants.

Together, these findings boost the known genetic causes of variation in the ability to smell odorants.

These findings present an opportunity to investigate whether genetic variants that affect the ability to smell food compounds also affect preferences for foods and beverages containing those compounds.

## PLENARY LECTURE II

### BITTER GUSTATORY RECEPTORS OF *DROSOPHILA MELANOGASTER*

**Aidan Kiely** and John R. Carlson

*Yale University, USA*

Animals use their sense of taste to determine the palatability and nutritive value of their food sources. One key aspect of the decision to ingest a food is the avoidance of toxic substances, which often have a bitter taste. A keen sense of bitter taste is therefore of great importance for the survival of an organism, though it remains unclear how the gustatory system encodes and discriminates bitter taste. *Drosophila melanogaster* has 60 gustatory receptor genes that encode 68 GR proteins, and recent research has identified that a subset of 38 of these are expressed in bitter sensitive neurons in the labellum. Electrophysiological investigation of these neurons using a panel of 16 bitter tastants has shown that there are four functional classes of bitter neurons. However, some neurons in these classes express as many as 28 GRs. This complexity makes the assignment of ligands to particular receptors difficult. To this end, we set out to develop a method to express and analyze the function of individual GRs. Here we present data showing that functional expression of GRs in insect and human cell lines can be improved by the co-expression of broadly expressed members of the bitter GR clade, and discuss some of the opportunities and challenges of this approach. Understanding of bitter taste coding may lead to the development of novel control agents for insect pest species.

### USE OF INSECT OLFACTORY SYSTEM AS A BIOSENSOR

**Kye Chung Park,** David Maxwell Suckling and Rachael Sagar

*The New Zealand Institute for Plant & Food Research Ltd, Lincoln, New Zealand*

Insects have well-developed olfactory sensory systems with high sensitivity and specificity, as a result of long evolutionary processes between insects and their hosts. The olfactory responses of insects to specific odorants can be monitored by using various techniques ranging molecular to behavioural levels. The electrophysiological recording techniques, such as electroantennogram (EAG), gas chromatograph-linked EAG (GC-EAD) and single sensillum recording, to monitor the activities of the olfactory sensory neurons in insects have been powerful tools to identify numerous semiochemicals. These techniques have been further developed to be used as a hybrid olfactory tissue-based biosensor for detecting volatile compounds of interest. Simultaneous signal monitoring from differentially tuned multiple antennae of different insect species enabled us to discriminate different volatile compounds in near-real time amongst mixtures of confluent odour filaments in a plume. The nanoscale structure and surface chemistry of the olfactory sensilla investigated with the atomic

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and chemical force microscopy suggests the selective and facilitated entry of particular types of odour molecules through the sensillum cuticle before reaching the receptors on the dendritic membrane. These techniques can be developed as a practical olfactory biosensor in many sectors including agricultural, medical, defence and biosecurity where the detection of low-level volatile compounds is critical. An example under development is the use of trained and restrained honeybees to detect signature compounds for tuberculosis, by proboscis extension response. In this case, the additional filter of behaviour is used in odorant screening.

Key words: biosensor, electrophysiology, honeybee, insect, olfaction, sensilla, tuberculosis

## TOWARDS A BIOSENSOR UTILISING HUMAN TASTE AND ODORANT RECEPTORS

**Seléne van der Poel[1],** Andrew V. Kralicek[1] and Richard D. Newcomb[1,2]

*[1] Molecular Sensing, Human Responses, Food Innovation, The New Zealand Institute for Plant & Food Research Limited, Auckland, New Zealand*

*[2] School of Biological Sciences, University of Auckland, Auckland , New Zealand*

We are endeavouring to build a biosensor using modified recombinant human chemosensory receptors. We propose to utilise Förster Resonance Energy Transfer (FRET) to monitor conformational changes in these receptors upon the binding of ligands. Human odorant and taste receptors are members of the G protein-coupled receptor (GPCR) superfamily of proteins. Studies with fluorescently labelled GPCRs reveal that the third intracellular loop and the C-terminus undergo substantial conformational changes upon ligand binding that can be quantified by FRET[1-3]. We will test the concept that FRET can be used to measure ligand detection using chemosensory receptors with three candidate modified odorant and taste receptors. We have chosen the human bitter taste receptor TAS2R38 and the human odorant receptors OR7D4 and OR2J3, all of which have well characterised ligands. All three receptors plus some control GPCRs were engineered to contain either Yellow Fluorescent Protein (YFP) or a binding site for the small fluorophore FlAsH (fluorescein biarsenical hairpin binder) in the third intracellular loop and Cyan Fluorescent Protein (CFP) at their C-terminus. The constructs were expressed in HEK293T cells or modified versions thereof to enhance plasma membrane expression. Levels of expression and localisation to the plasma membrane were determined by fluorescense microscopy and colocalisation with wheatgerm aglutatin. Preliminary experiments to detect changes in FRET upon the addition of ligand are underway.

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## MONITORING SMELL ADSORPTION INSIDE A BIOFILTER USING AN ELECTRONIC NOSE

**Graham Bell** and Larry Botham

*E-Nose Pty Ltd and Bioaction Pty Ltd*

Biofilters are commonly used to remove smell from polluted air generated by various industrial processes. Improvements are being made to filter container design and to the adsorptive materials used therein. Knowing how well the biofilter performs and when the material requires refreshing can save an industrial operation time and money and, particularly, pressure from communities and environmental protection agencies when the filter ceases to work effectively. A biofilter designed by Bioaction Pty Ltd and trialled at the organic fertilizer plant of Yates Pty Ltd, Wyee, NSW. It allowed measurement to be made at six levels of depth in the filter bed as well as at the inlet and outlet. An E-Nose Mk4 was used to record quality and quantity of odorous molecules over a period of several minutes at each depth. Most smelly compounds entering the filter bed were removed between level 6 (nearest inlet) and level 4 (about midway into the filter). This shows that the deeper material is doing most of the work and is likely to become exhausted before the upper layers. The combination of E-nose and filter design tells us what the optimal effective depth is for material in the filter, what material needs changing and when, what materials work best, and provides guidance on what bacterial processes might be occurring at various depths. By knowing what is happening inside the filter in real time, biofilters will play a more effective role in minimising fugitive odours of concern to communities.

## TOPOLOGY AND FUNCTIONAL ANALYSIS OF BMGR8, A *BOMBYX MORI* GUSTATORY RECEPTOR

Hui-Jie Zhang[1,3], Alisha R. Anderson[2,3], **Stephen C. Trowell[2,3],** A-Rong Luo[3], Zhong-Huai Xiang[1], Qing-You Xia[1,4]

*Stephen.Trowell@csiro.au*

*[1] The Key Sericultural Laboratory of Agricultural Ministry, Southwest University, Chongqing 400715, China*

*[2] CSIRO Food Futures Flagship*

*[3] CSIRO Ecosystem Sciences, GPO Box 1700, Canberra, ACT 2601, Australia*

*[4] Institute of Agronomy and Life Science, Chongqing University, Chongqing 400044, China*

"Sugar" and "bitter" receptors comprise two large clades in insect gustatory receptor (GR) phylogeny. Based on homologies with identified receptors, we identified five putative sugar receptors and a large number of putative bitter receptors from the genome of the lepidopteran *Bombyx mori*.

Despite almost 60 putative sugar GRs being identified across all sequenced insect genomes, ligands have only been assigned to a few of the *Drosophila* and one of the *B. mori* receptor sequences. BmGr8, a silkmoth gustatory receptor from the sugar receptor subfamily, was expressed in insect cells. Functional analysis, using a modified calcium-imaging assay, showed that BmGr8 can function independently in Sf9 cells and that it responds specifically and in a concentration-dependent manner to inositol, an essential nutrient for *B. mori*. The selectivity of BmGr8 responses is consistent with the known responses of one of the gustatory receptor neurons in the lateral styloconic sensilla of this species.

Insect gustatory receptors are predicted to have seven-transmembrane domains. They are distantly related to insect olfactory receptors, which have an inverted topology compared with G-protein coupled receptors. We have now determined that the transmembrane topology of BmGr8 is the same as insect olfactory receptors and different from mammalian olfactory and gustatory reeptors. We also found that an orphan receptor from the 'bitter' receptor subfamily, BmGr53, has the same topology as BmGr8. This is the first time that the topology of any insect gustatory receptor has been determined.

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## SEX-BIASED TRANSCRIPTION OF OLFACTORY RECEPTORS OF THE BEETLE *TRIBOLIUM CASTANEUM*

**Kelly Hill [1,2],** Bradley J. Stevenson [1,3], Alisha Anderson [3], Richard V. Glatz [2], and Sylwek Chyb [3]

*[1] Cooperative Research Centre for National Plant Biosecurity*

*[2] South Australian Research and Development Institute, Urrbrae, South Australia*

*[3] CSIRO Food Futures Flagship and Division of Ecosystem Sciences, Canberra, ACT 2601*

Stored product insect pests, such as the red flour beetle *(Tribolium castaneum)*, use their olfactory network to detect volatile chemicals with high sensitivity to perform a range of ecological functions such as finding mates, indicating overcrowding and finding suitable food sources. The sensitivity and specificity of their olfactory system relies on odourant receptors (ORs). For grain-infesting beetles like *Tribolium*, infestations are currently assessed by visual inspection of grain samples, which can be unreliable. We therefore aim to determine which receptors the beetles use to find each other and

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investigate the viability on integrating these receptors into a commercial sensing system capable of detecting low concentrations of compounds produced by the beetles.

One approach we are taking focuses on sex pheromone communication. We hypothesise sex pheromone receptors will show sex-biased transcription and are therefore comparing individual OR transcription in male and female beetles. Real-time quantitative PCR has uncovered two male-specific ORs and these receptors are targets for further functional investigations. Cell-based calcium assays are one of the methods we are using to test the hypothesis that the two sexually-biased receptors we have found, are involved in pheromone communication. We are investigating the specificity and sensitivity of ligand-induced receptor activation using a known pheromone of the beetle, 4,8-dimethyldecanal. So far, results indicate a response in cells expressing one of the two ORs. It is envisioned that research into insect pheromone receptor systems could not only have applications in sensing devices, but also potentially aid in development or refinement of integrated control measures.

### OLFACTORY RECEPTOR GENES FROM COTTON BOLLWORM *HELICOVERPA ARMIGERA*

**Wei Xu,** Alexie Papanicolaou and Alisha Anderson

*CSIRO Ecosystem Sciences, Black Mountain, ACT, Australia 2601*

Much insect behaviour is guided by olfactory system. Insect odorant receptors (ORs), which are expressed in olfactory receptor neurons (ORNs) and housed in olfactory sensilla, are potential molecular targets for the development of novel insect attractants and repellents. Here we identified OR genes from polyphagous pest, *Helicoverpa armigera* transcriptome sequences. The expression profiles of OR genes were analysed among different tissues, ages and between the sexes. The result showed most OR genes are primarily expressed in male adult heads, female adult heads as well as larvae antennae. Orco, the essential subunit for the odorant receptors, is highly expressed in both male and female heads and larvae antennae. OR13, the potential sex pheromone receptor, is only highly expressed in male heads but not female. This study will help us build connections between the OR genes and their functions, leading to a better understanding of the molecular mechanism of polyphagous pest olfactory system and assist in the development of environmentally friendly insect control strategies.

Keywords: olfaction, transcriptome, olfactory receptors, cotton bollworm, *Helicoverpa armigera*

### IDENTIFICATION OF CANDIDATE PHEROMONE RECEPTORS FROM THE LIGHT BROWN APPLE MOTH (*EPIPHYAS POSTVITTANA*)

**Melissa D. Jordan[1],** Jacob A. Corcoran[1,2], Doreen S. Begum[1,2], Richard D. Newcomb[1,2]

*[1]. The New Zealand Institute for Plant & Food Research Limited, Auckland, New Zealand*

*[2]. School of Biological Sciences, University of Auckland, Auckland, New Zealand*

*Epiphyas postvittana*, or the light brown apple moth (LBAM), is a common horticultural pest that causes damage in many important horticultural crops of Australia, New Zealand and most recently California. Control strategies employed to manage this pest include the use of insecticides, biological control agents and mating disruption. We aim to improve the efficacy of mating disruption using knowledge of the molecular basis of the insect's sex pheromone reception system.

Expressed sequence tag and genome databases have been established from which we have isolated odorant (OR) and pheromone (PR) receptor genes. Using comparison with known insect ORs and PRs we have identified fifty-two putative receptors from LBAM. Quantitative real-time polymerase chain reaction was used to determine the relative abundance of their transcripts in a range of tissues including male and female antennae. We found six transcripts displaying male-biased expression in antennae, which likely indicates a role in the detection of the female-produced sex pheromone. Full-length sequences of the male biased LBAM receptors are being generated and will be introduced into cell-based reporter systems to facilitate the characterization of their interactions with LBAM sex pheromone components.

### IDENTIFICATION OF CANDIDATE PHEROMONE RECEPTORS FROM NEW ZEALAND ENDEMIC LEAFROLLER MOTHS

**Bernd Steinwender[1,2,3],** Thomas Buckley[3,4], Richard D. Newcomb[1,2,3]

*[1] The New Zealand Institute for Plant & Food Research, Auckland, New Zealand, presenting Author*

*[2] School of Biological Sciences, University of Auckland, Auckland, New Zealand*

*[3] Allan Wilson Centre for Molecular Ecology and Evolution*

*[4] Landcare Research, Auckland, New Zealand*

The major feature of mate recognition in moths is the ability of the male to distinguish between sex pheromone blends of their own species and other, often closely related, species. Different pheromone components are detected by the male by pheromone receptors in sensilla on their antennae. We are investigating whether mutations of pheromone receptor genes in sibling species pairs within the New Zealand endemic leafroller genera *Ctenopseustis* and *Planotortrix* have altered their sex pheromone reception. Fifty-two putative odorant receptor genes have already been identified in the closely related tortricid moth, *Epiphyas postvittana*. These sequences were blasted against preliminary genome assembles of *C. obliquana* and *P. octo* to find orthologous receptor genes. Levels of expression in antennae were assessed for each candidate receptor gene to test for sex-biased expression, as sex pheromone receptors are assumed to be more highly expressed in the antennae of males compared with females. For *C. obliquana* 26 putative odorant receptor genes could be identified, with five showing male-biased and four female-biased expression. In *C. herana* 18 putative odorant receptor genes were identified so far, two male-biased and three female-biased. Both male-biased odorant receptor genes in *C. herana* are also more highly expressed in males than in females of *C. obliquana*. Two out of the three odorant receptor genes in *C. herana* females also show higher expression in female *C. obliquana*; one receptor gene is female-biased solely in *C. herana*. Future work involves conducting a similar analysis of expression of odorant receptor genes from the two *Planotortrix* species, *P. octo* and *P. excessana*. Following that, we will isolate full-length cDNAs for receptors showing male-biased expression from all four species to allow comparison of their coding sequences and functional evaluation using cell-based assays ■

# Upcoming Events

| | |
|---|---|
| 12-17 January 2012 | **ISOCS Short Course (Winter)**<br>Data Analysis, Robotics, & Mobile Applications of Chemical Sensors<br>Kuhtai, Austria<br>www.olfactionsociety.org |
| 29 January – 1 February 2012 | **Australian Neuroscience Society (ANS)**<br>Annual Conference<br>Gold Coast, Queensland<br>www.ans.org.au |
| 7-9 March 2012 | **EcoForum**<br>Conference and Exhibition<br>Australian Technology Park, Sydney<br>www.ecoforum.net.au |
| 25-28 April 2012 | **AChemS XXXIV**<br>Hyatt Huntington Beach, California<br>www.achems.org |
| 20-23 May 2012 | **14th International Meeting on Chemical Sensors**<br>Nuremberg, Germany<br>www.icms2012.de |
| 3-8 June 2012 | **School on the Senses: Neurobiology & Behaviour**<br>Bertinoro, Italy<br>kathyann.koralek@gmail.com |
| 4-7 June 2012 | **ISOCS Short Course (Summer)**<br>Nanotechnologies for Chemical Trace Detection<br>Tarragona, Spain<br>www.olfactionsociety.org |
| 23-27 June 2012 | **ISOT 2012 (with ECRO XXII)**<br>Stockholm, Sweden<br>administration@ISOTXVI.com |
| 10-13 July 2012 | **11th Sensometric Meeting**<br>Rennes, France<br>www.sensometric.org |

**ChemoSense** (ISSN 1442-9098)
Web: chemosense.info
Published by **E-Nose Pty Ltd**
P.O. Box 488 Gladesville, NSW Australia 2111
Ph. (+61 2) 9209 4083 ; Fax (+61 2) 9209 4081

**Production Team**
**Editor & Advertising:** Graham Bell, g.bell@e-nose.info
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